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Diagnostics and therapeutics for diseases associated with protein kinase, cGMP-dependent type 1 (PRKG1)

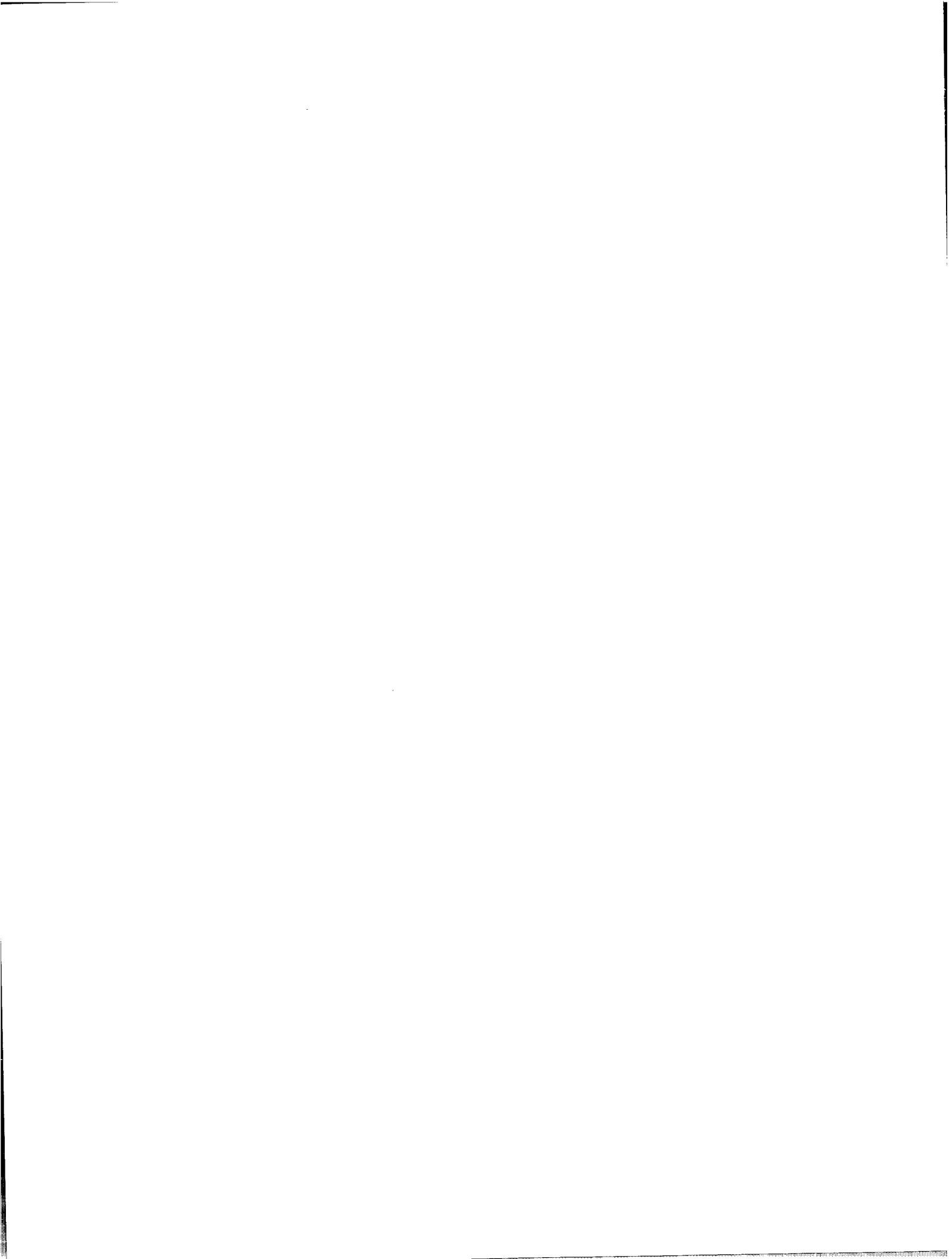
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Diagnostics and Therapeutics for Diseases Associated with Protein Kinase, cGMP-Dependent, Type I (PRKG1)

Technical field of the invention

The present invention is in the field of molecular biology, more particularly, the present invention 5 relates to nucleic acid sequences and amino acid sequences of a human PRKG1 and its regulation for the treatment of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases in mammals.

10 Background of the invention

PRKG1 is a member of the enzyme group of kinases [Sandberg et al. (1989), Tamura et al. (1996), Orstavik et al. (1997), Li et al. (2003), Pfeifer et al. (1998), Libersan et al. (2003), Airhart et al. (2003), WO0175067, WO0255664, WO9845704]. Kinases are enzymes, which catalyse the transfer of a phosphate group (phosphorylation) from a donor (mainly ATP) onto an acceptor 15 molecule's nucleophilic functional group, such as hydroxy-, carboxy-, guanidino-, thiol-, or imidazole- groups. Kinases can act on a variety of molecules like small metabolites, or proteins (protein kinases).

Kinases that phosphorylate small organic molecules often play important roles in metabolic pathways such as glycolysis (e.g. hexokinase, glucokinase, or phosphofructokinase), or in anabolic 20 pathways.

Protein kinases play critical roles in cellular signal transduction.

Cellular signal transduction is a fundamental mechanism whereby extracellular stimuli are relayed to the interior of cells and subsequently regulate diverse cellular processes. One of the key biochemical mechanisms of signal transduction involves the reversible phosphorylation of 25 proteins. Phosphorylation of polypeptides regulates the activity of mature proteins by altering their structure and function. Phosphate most often resides on the hydroxyl moiety (--OH) of serine, threonine, or tyrosine amino acids in proteins.

Kinases regulate many different cell proliferation, differentiation, and signaling processes by adding phosphate groups to proteins. Uncontrolled signaling has been implicated in a variety of 30 disease conditions including inflammation, cancer, arteriosclerosis, and psoriasis. Reversible protein phosphorylation is the main strategy for controlling activities of eukaryotic cells. The high

energy phosphate, which drives activation, is generally transferred from adenosine triphosphate molecules (ATP) to a particular protein by protein kinases and removed from that protein by protein phosphatases. Phosphorylation occurs in response to extracellular signals (hormones, neurotransmitters, growth and differentiation factors, etc), cell cycle checkpoints, and environmental or nutritional stresses and is roughly analogous to turning on a molecular switch. When the switch goes on, the appropriate protein kinase activates a metabolic enzyme, regulatory protein, receptor, cytoskeletal protein, ion channel or pump, or transcription factor.

The kinases comprise the largest known protein group, a superfamily of enzymes with widely varied functions and specificities. They are usually named after their substrate, their regulatory molecules, or some aspect of a mutant phenotype. With regard to substrates, the protein kinases may be roughly divided into two groups; those that phosphorylate tyrosine residues (protein tyrosine kinases, PTK) and those that phosphorylate serine or threonine residues (serine/threonine kinases, STK). A few protein kinases have dual specificity and phosphorylate threonine and tyrosine residues. Almost all kinases contain a similar 250-300 amino acid catalytic domain. The N-terminal domain, which contains subdomains I-IV, generally folds into a two-lobed structure, which binds and orients the ATP (or GTP) donor molecule. The larger C terminal lobe, which contains subdomains VI-XI, binds the protein substrate and carries out the transfer of the gamma phosphate from ATP to the hydroxyl group of a serine, threonine, or tyrosine residue. Subdomain V spans the two lobes.

The kinases may be categorized into families by the different amino acid sequences (generally between 5 and 100 residues) located on either side of, or inserted into loops of, the kinase domain. These added amino acid sequences allow the regulation of each kinase as it recognizes and interacts with its target protein. The primary structure of the kinase domains is conserved and can be further subdivided into 11 subdomains. Each of the 11 subdomains contains specific residues and motifs or patterns of amino acids that are characteristic of that subdomain and are highly conserved [Hardie, G. and Hanks, S. (1995)].

The second messenger dependent protein kinases primarily mediate the effects of second messengers such as cyclic AMP (cAMP), cyclic GMP, inositol triphosphate, phosphatidylinositol, 3,4,5-triphosphate, cyclic-ADPribose, arachidonic acid, diacylglycerol and calcium-calmodulin. The cyclic-AMP dependent protein kinases (PKA) are important members of the STK family. Cyclic-AMP is an intracellular mediator of hormone action in all prokaryotic and animal cells that have been studied. Such hormone-induced cellular responses include thyroid hormone secretion, cortisol secretion, progesterone secretion, glycogen breakdown, bone resorption, and regulation of heart rate and force of heart muscle contraction. PKA is found in all animal cells and is thought to

account for the effects of cyclic-AMP in most of these cells. Altered PKA expression is implicated in a variety of disorders and diseases including cancer, thyroid disorders, diabetes, atherosclerosis, and cardiovascular disease [Isselbacher, K. J. et al. (1994)].

Calcium-calmodulin (CaM) dependent protein kinases are also members of STK family.

5 Calmodulin is a calcium receptor that mediates many calcium regulated processes by binding to target proteins in response to the binding of calcium. The principle target protein in these processes is CaM dependent protein kinases. CaM-kinases are involved in regulation of smooth muscle contraction (MLC kinase), glycogen breakdown (phosphorylase kinase), and neurotransmission (CaM kinase I and CaM kinase II). CaM kinase I phosphorylates a variety of

10 substrates including the neurotransmitter related proteins synapsin I and II, the gene transcription regulator, CREB, and the cystic fibrosis conductance regulator protein, CFTR [Haribabu, B. et al. (1995)]. CaM II kinase also phosphorylates synapsin at different sites, and controls the synthesis of catecholamines in the brain through phosphorylation and activation of tyrosine hydroxylase. Many of the CaM kinases are activated by phosphorylation in addition to binding to CaM. The kinase

15 may autophosphorylate itself, or be phosphorylated by another kinase as part of a "kinase cascade".

Another ligand-activated protein kinase is 5'-AMP-activated protein kinase (AMPK) [Gao, G. et al. (1996)]. Mammalian AMPK is a regulator of fatty acid and sterol synthesis through phosphorylation of the enzymes acetyl-CoA carboxylase and hydroxymethylglutaryl-CoA reductase

20 and mediates responses of these pathways to cellular stresses such as heat shock and depletion of glucose and ATP. AMPK is a heterotrimeric complex comprised of a catalytic alpha subunit and two non-catalytic beta and gamma subunits that are believed to regulate the activity of the alpha subunit. Subunits of AMPK have a much wider distribution in non-lipogenic tissues such as brain, heart, spleen, and lung than expected. This distribution suggests that its role may extend beyond

25 regulation of lipid metabolism alone.

The mitogen-activated protein kinases (MAP) are also members of the STK family. MAP kinases also regulate intracellular signaling pathways. They mediate signal transduction from the cell surface to the nucleus via phosphorylation cascades. Several subgroups have been identified, and each manifests different substrate specificities and responds to distinct extracellular stimuli [Egan, S. E. and Weinberg, R. A. (1993)]. MAP kinase signaling pathways are present in mammalian cells as well as in yeast. The extracellular stimuli that activate mammalian pathways include epidermal growth factor (EGF), ultraviolet light, hyperosmolar medium, heat shock, endotoxic lipopolysaccharide (LPS), and pro-inflammatory cytokines such as tumor necrosis factor (TNF) and interleukin-1 (IL-1).

PRK (proliferation-related kinase) is a serum/cytokine inducible STK that is involved in regulation of the cell cycle and cell proliferation in human megakaryocytic cells [Li, B. et al. (1996)]. PRK is related to the polo (derived from humans polo gene) family of STKs implicated in cell division. PRK is downregulated in lung tumor tissue and may be a proto-oncogene whose deregulated expression in normal tissue leads to oncogenic transformation. Altered MAP kinase expression is implicated in a variety of disease conditions including cancer, inflammation, immune disorders, and disorders affecting growth and development.

The cyclin-dependent protein kinases (CDKs) are another group of STKs that control the progression of cells through the cell cycle. Cyclins are small regulatory proteins that act by binding to and activating CDKs that then trigger various phases of the cell cycle by phosphorylating and activating selected proteins involved in the mitotic process. CDKs are unique in that they require multiple inputs to become activated. In addition to the binding of cyclin, CDK activation requires the phosphorylation of a specific threonine residue and the dephosphorylation of a specific tyrosine residue.

Protein tyrosine kinases, PTKs, specifically phosphorylate tyrosine residues on their target proteins and may be divided into transmembrane, receptor PTKs and nontransmembrane, non-receptor PTKs. Transmembrane protein-tyrosine kinases are receptors for most growth factors. Binding of growth factor to the receptor activates the transfer of a phosphate group from ATP to selected tyrosine side chains of the receptor and other specific proteins. Growth factors (GF) associated with receptor PTKs include; epidermal GF, platelet-derived GF, fibroblast GF, hepatocyte GF, insulin and insulin-like GFs, nerve GF, vascular endothelial GF, and macrophage colony stimulating factor.

Non-receptor PTKs lack transmembrane regions and, instead, form complexes with the intracellular regions of cell surface receptors. Such receptors that function through non-receptor PTKs include those for cytokines, hormones (growth hormone and prolactin) and antigen-specific receptors on T and B lymphocytes.

In an effort to discover novel treatments for diseases, biomedical researchers and chemists have designed, synthesized, and tested molecules that inhibit the function of protein kinases. Some small organic molecules form a class of compounds that modulate the function of protein kinases. The modulatory compounds are potentially advantageous therapeutics for disease conditions including but not limited to inflammation, cancer, arteriosclerosis, and psoriasis.

TaqMan-Technology / expression profiling

TaqMan is a recently developed technique, in which the release of a fluorescent reporter dye from a hybridisation probe in real-time during a polymerase chain reaction (PCR) is proportional to the accumulation of the PCR product. Quantification is based on the early, linear part of the reaction,
5 and by determining the threshold cycle (CT), at which fluorescence above background is first detected.

Gene expression technologies may be useful in several areas of drug discovery and development, such as target identification, lead optimization, and identification of mechanisms of action. The TaqMan technology can be used to compare differences between expression profiles of normal
10 tissue and diseased tissue. Expression profiling has been used in identifying genes, which are up- or downregulated in a variety of diseases. An interesting application of expression profiling is temporal monitoring of changes in gene expression during disease progression and drug treatment or in patients versus healthy individuals. The premise in this approach is that changes in pattern of gene expression in response to physiological or environmental stimuli (e.g., drugs) may serve as
15 indirect clues about disease-causing genes or drug targets. Moreover, the effects of drugs with established efficacy on global gene expression patterns may provide a guidepost, or a genetic signature, against which a new drug candidate can be compared.

PRKG1

The nucleotide sequence of PRKG1 is accessible in public databases by the accession number
20 NM_006258 and is given in SEQ ID NO: 1. The amino acid sequence of PRKG1 is depicted in SEQ ID NO: 2.

Cyclic GMP and cyclic GMP-dependent protein kinase play important roles in physiologic processes such as relaxation of vascular smooth muscle and inhibition of platelet aggregation. Two main forms of cGK have been identified: a soluble form designated type I and an intrinsic
25 membrane-bound form designated type II. Sandberg et al. (1989) isolated and characterized cDNA clones for the type I beta isozyme from human placenta libraries.

Tamura et al. (1996) cloned a human cGKI-alpha cDNA by RT-PCR of aorta RNA using primers based on the sequence of a bovine cGKI-alpha cDNA. The predicted 671-amino acid human cGKI-alpha protein is nearly identical to bovine cGKI-alpha. Based on Southern blot and sequence analyses, Tamura et al. (1996) suggested that cGKI-alpha and cGKI-beta (=PRKG1) are generated by alternative splicing of a single gene. By Northern blot analysis, cGKI-alpha was abundantly
30

expressed as a 7.0-kb mRNA in aorta, heart, kidneys and adrenals; the 7.0-kb cGKI-beta (=PRKG1) mRNA was abundantly expressed only in the uterus.

Orstavik et al. (1997) noted that type I cGK is a homodimer, with each monomer containing a regulatory cGMP-binding domain and a catalytic domain. By Northern blot analysis, they showed
5 that type I cGK-alpha mRNA was most abundant in lung and placenta, while type I cGK-beta (=PRKG1) was expressed at highest levels in bladder, uterus, adrenal gland, and fallopian tube.

Li et al. (2003) showed that PRKG1 plays an important stimulatory role in platelet activation. Expression of recombinant PRKG1 in a reconstituted cell model enhanced von Willebrand factor (VWF)-induced activation of the platelet integrin alpha-IIb/beta-3. Prkg1 knockout mice showed
10 impaired platelet responses to VWF or low doses of thrombin and prolonged bleeding time. Human platelet aggregation induced by VWF or low-dose thrombin was inhibited by PRKG1 inhibitors but enhanced by cGMP. Furthermore, a cGMP-enhancing agent, sildenafil, promoted VWF- or thrombin-induced platelet aggregation. The cGMP-stimulated platelet responses were biphasic, consisting of an initial transient stimulatory response that promoted platelet aggregation
15 and a subsequent inhibitory response that limited the size of thrombi.

Orstavik et al. (1997) reported that the type I PRKG1 gene consists of 19 exons spanning at least 220 kb. The first 2 exons, which the authors called 1-alpha and 1-beta, are used alternatively and encode the alpha isoform- and beta isoform-specific sequences. Orstavik et al. (1997) noted that 5 of the 7 splice sites in the *Drosophila melanogaster* DG2 gene, which encodes a PRKG1, are also
20 present in the human type I PRKG1 gene. They reported that levels of the DG2-encoded PRKG1 in *Drosophila* affect food-search behavior and account for a naturally occurring behavioral polymorphism.

Pfeifer et al. (1998) generated mice deficient in PRKG1 by targeted disruption. Loss of PRKG1 abolished nitric oxide/cGMP-dependent relaxation of smooth muscle, resulting in severe
25 vascular and intestinal dysfunction. However, PRKG1-deficient smooth muscle responded normally to cAMP, indicating that cAMP and cGMP signal via independent pathways, with PRKG1 being the specific mediator of the nitric oxide/cGMP effects in murine smooth muscle.

P-selectin is rapidly translocated from platelet alpha-granules following activation. Intracellular cyclic AMP (cAMP) is a potent inhibitory pathway that results in global downregulation of
30 platelet activation. While cAMP-dependent protein kinase (PKA) has long been considered as the main mediator of cAMP-dependent effects, no study has yet evaluated its effect on P-selectin expression in human platelets. Pretreatment of thrombin-stimulated platelets with forskolin resulted in a concentration- dependent inhibition of P-selectin expression that correlated with

adenylyl cyclase activity. Inhibition of PKA with H-89 reversed cAMP-induced inhibition of P-selectin while cGMP-dependent protein kinase (PKG) inhibition with KT5823 significantly potentiated cAMP-dependent inhibition of P-selectin. Similar results were also observed in a platelet/neutrophil binding assay. In conclusion, cAMP-induced inhibition of P-selectin expression
5 is, in large part, mediated through activation of PKA. PKG appears to be solicited for P-selectin expression when cAMP levels are elevated which suggest a cAMP/PKG-dependent pathway of platelet activation [Libersan et al. (2003)].

Circulating natriuretic peptides such as atrial natriuretic peptide (ANP) counterbalance the effects of hypertension and inhibit cardiac hypertrophy by activating cGMP-dependent protein kinase
10 (PKG). Natriuretic peptide binding to type I receptors (NPRA and NPRB) activates their intrinsic guanylyl cyclase activity, resulting in a rapid increase in cytosolic cGMP that subsequently activates PKG. Phosphorylation of the receptor by an unknown serine/threonine kinase is required before ligand binding can activate the cyclase. While searching for downstream PKG partners using a yeast two-hybrid screen of a human heart cDNA library, Airhart

15 et al. (2003) unexpectedly found an upstream association with NPRA. PKG is a serine/threonine kinase capable of phosphorylating NPRA in vitro; however, regulation of NPRA by PKG has not been previously reported. Airhart et al. (2003) show that PKG is recruited to the plasma membrane following ANP treatment, an effect that can be blocked by pharmacological inhibition of PKG activation. Furthermore, PKG participates in a ligand-dependent gain-of-function loop that
20 significantly increases the intrinsic cyclase activity of the receptor. PKG translocation is ANP-dependent but not nitric oxide-dependent. Results by Airhart et al. (2003) suggest that anchoring of PKG to NPRA is a key event after ligand binding that determines distal effects. As such, the NPRA-PKG association may represent a novel mechanism for compartmentation of cGMP-mediated signaling and regulation of receptor sensitivity

25 PRKG1 is published in WO0175067, WO0255664 and WO9845704.

PRKG1 shows the highest homology (48%) to the human protein kinase, cGMP-dependent, type II as shown in example 1.

Summary of the invention

The invention relates to novel disease associations of PRKG1 polypeptides and polynucleotides.

30 The invention also relates to novel methods of screening for therapeutic agents for the treatment of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases,

muscle-skeleton-diseases, neurological diseases and urological diseases in a mammal. The invention also relates to pharmaceutical compositions for the treatment of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-
5 diseases, neurological diseases and urological diseases in a mammal comprising a PRKG1 polypeptide, a PRKG1 polynucleotide, or regulators of PRKG1 or modulators of PRKG1 activity. The invention further comprises methods of diagnosing cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastro-
10 enterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases in a mammal.

Brief Description of the Drawings

Fig. 1 shows the nucleotide sequence of a PRKG1 polynucleotide (SEQ ID NO: 1).

Fig. 2 shows the amino acid sequence of a PRKG1 polypeptide (SEQ ID NO: 2).

Fig. 3 shows the nucleotide sequence of a primer useful for the invention (SEQ ID NO: 3).

15 Fig. 4 shows the nucleotide sequence of a primer useful for the invention (SEQ ID NO: 4).

Fig. 5 shows a nucleotide sequence useful as a probe to detect proteins of the invention (SEQ ID NO: 5).

Detailed description of the invention

Definition of terms

20 An “oligonucleotide” is a stretch of nucleotide residues which has a sufficient number of bases to be used as an oligomer, amplimer or probe in a polymerase chain reaction (PCR). Oligonucleotides are prepared from genomic or cDNA sequence and are used to amplify, reveal, or confirm the presence of a similar DNA or RNA in a particular cell or tissue. Oligonucleotides or oligomers comprise portions of a DNA sequence having at least about 10 nucleotides and as many
25 as about 35 nucleotides, preferably about 25 nucleotides.

“Probes” may be derived from naturally occurring or recombinant single- or double-stranded nucleic acids or may be chemically synthesized. They are useful in detecting the presence of identical or similar sequences. Such probes may be labeled with reporter molecules using nick translation, Klenow fill-in reaction, PCR or other methods well known in the art. Nucleic acid

probes may be used in southern, northern or in situ hybridizations to determine whether DNA or RNA encoding a certain protein is present in a cell type, tissue, or organ.

A “fragment of a polynucleotide“ is a nucleic acid that comprises all or any part of a given nucleotide molecule, the fragment having fewer nucleotides than about 6 kb, preferably fewer than

5 about 1 kb.

“Reporter molecules“ are radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents which associate with a particular nucleotide or amino acid sequence, thereby establishing the presence of a certain sequence, or allowing for the quantification of a certain sequence.

“Chimeric“ molecules may be constructed by introducing all or part of the nucleotide sequence of

10 this invention into a vector containing additional nucleic acid sequence which might be expected to change any one or several of the following PRKG1 characteristics: cellular location, distribution, ligand-binding affinities, interchain affinities, degradation/turnover rate, signaling, etc.

“Active“, with respect to a PRKG1 polypeptide, refers to those forms, fragments, or domains of a PRKG1 polypeptide which retain the biological and/or antigenic activity of a PRKG1 polypeptide.

15 “Naturally occurring PRKG1 polypeptide“ refers to a polypeptide produced by cells which have not been genetically engineered and specifically contemplates various polypeptides arising from post-translational modifications of the polypeptide including but not limited to acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation.

20 “Derivative“ refers to polypeptides which have been chemically modified by techniques such as ubiquitination, labeling (see above), pegylation (derivatization with polyethylene glycol), and chemical insertion or substitution of amino acids such as ornithine which do not normally occur in human proteins.

25 “Conservative amino acid substitutions“ result from replacing one amino acid with another having similar structural and/or chemical properties, such as the replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine.

“Insertions“ or “deletions“ are typically in the range of about 1 to 5 amino acids. The variation allowed may be experimentally determined by producing the peptide synthetically while systematically making insertions, deletions, or substitutions of nucleotides in the sequence using recombinant DNA techniques.

A "signal sequence" or "leader sequence" can be used, when desired, to direct the polypeptide through a membrane of a cell. Such a sequence may be naturally present on the polypeptides of the present invention or provided from heterologous sources by recombinant DNA techniques.

An "oligopeptide" is a short stretch of amino acid residues and may be expressed from an 5 oligonucleotide. Oligopeptides comprise a stretch of amino acid residues of at least 3, 5, 10 amino acids and at most 10, 15, 25 amino acids, typically of at least 9 to 13 amino acids, and of sufficient length to display biological and/or antigenic activity.

"Inhibitor" is any substance which retards or prevents a chemical or physiological reaction or response. Common inhibitors include but are not limited to antisense molecules, antibodies, and 10 antagonists.

"Standard expression" is a quantitative or qualitative measurement for comparison. It is based on a statistically appropriate number of normal samples and is created to use as a basis of comparison when performing diagnostic assays, running clinical trials, or following patient treatment profiles.

"Animal" as used herein may be defined to include human, domestic (e.g., cats, dogs, etc.), 15 agricultural (e.g., cows, horses, sheep, etc.) or test species (e.g., mouse, rat, rabbit, etc.).

A "PRKG1 polynucleotide", within the meaning of the invention, shall be understood as being a nucleic acid molecule selected from a group consisting of

- (i) nucleic acid molecules encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 2,
- 20 (ii) nucleic acid molecules comprising the sequence of SEQ ID NO: 1,
- (iii) nucleic acid molecules having the sequence of SEQ ID NO: 1,
- (iv) nucleic acid molecules the complementary strand of which hybridizes under stringent conditions to a nucleic acid molecule of (i), (ii), or (iii); and
- (v) nucleic acid molecules the sequence of which differs from the sequence of a nucleic acid 25 molecule of (iii) due to the degeneracy of the genetic code;

wherein the polypeptide encoded by said nucleic acid molecule has PRKG1 activity.

A "PRKG1 polypeptide", within the meaning of the invention, shall be understood as being a polypeptide selected from a group consisting of

- (i) polypeptides having the sequence of SEQ ID NO: 2,
- (ii) polypeptides comprising the sequence of SEQ ID NO: 2,
- (iii) polypeptides encoded by PRKG1 polynucleotides; and
- (iv) polypeptides which show at least 99%, 98%, 95%, 90%, or 80% homology with a
5 polypeptide of (i), (ii), or (iii);

wherein said polypeptide has PRKG1 activity.

The nucleotide sequences encoding a PRKG1 (or their complement) have numerous applications in techniques known to those skilled in the art of molecular biology. These techniques include use as hybridization probes, use in the construction of oligomers for PCR, use for chromosome and
10 gene mapping, use in the recombinant production of PRKG1, and use in generation of antisense DNA or RNA, their chemical analogs and the like. Uses of nucleotides encoding a PRKG1 disclosed herein are exemplary of known techniques and are not intended to limit their use in any technique known to a person of ordinary skill in the art. Furthermore, the nucleotide sequences disclosed herein may be used in molecular biology techniques that have not yet been developed,
15 provided the new techniques rely on properties of nucleotide sequences that are currently known, e.g., the triplet genetic code, specific base pair interactions, etc.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of PRKG1 - encoding nucleotide sequences may be produced. Some of these will only bear minimal homology to the nucleotide sequence of the known and naturally occurring

20 PRKG1. The invention has specifically contemplated each and every possible variation of nucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the nucleotide sequence of naturally occurring PRKG1, and all such variations are to be considered as being specifically disclosed.

25 Although the nucleotide sequences which encode a PRKG1, its derivatives or its variants are preferably capable of hybridizing to the nucleotide sequence of the naturally occurring PRKG1 polynucleotide under stringent conditions, it may be advantageous to produce nucleotide sequences encoding PRKG1 polypeptides or its derivatives possessing a substantially different codon usage. Codons can be selected to increase the rate at which expression of the peptide occurs

30 in a particular prokaryotic or eukaryotic expression host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding a PRKG1 polypeptide and/or its derivatives without altering the

encoded amino acid sequence include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

Nucleotide sequences encoding a PRKG1 polypeptide may be joined to a variety of other nucleotide sequences by means of well established recombinant DNA techniques. Useful nucleotide sequences for joining to PRKG1 polynucleotides include an assortment of cloning vectors such as plasmids, cosmids, lambda phage derivatives, phagemids, and the like. Vectors of interest include expression vectors, replication vectors, probe generation vectors, sequencing vectors, etc. In general, vectors of interest may contain an origin of replication functional in at least one organism, convenient restriction endonuclease sensitive sites, and selectable markers for one or more host cell systems.

Another aspect of the subject invention is to provide for PRKG1-specific hybridization probes capable of hybridizing with naturally occurring nucleotide sequences encoding PRKG1. Such probes may also be used for the detection of similar kinase encoding sequences and should preferably show at least 40% nucleotide identity to PRKG1 polynucleotides. The hybridization probes of the subject invention may be derived from the nucleotide sequence presented as SEQ ID NO: 1 or from genomic sequences including promoter, enhancers or introns of the native gene. Hybridization probes may be labelled by a variety of reporter molecules using techniques well known in the art.

It will be recognized that many deletional or mutational analogs of PRKG1 polynucleotides will be effective hybridization probes for PRKG1 polynucleotides. Accordingly, the invention relates to nucleic acid sequences that hybridize with such PRKG1 encoding nucleic acid sequences under stringent conditions.

“Stringent conditions” refers to conditions that allow for the hybridization of substantially related nucleic acid sequences. For instance, such conditions will generally allow hybridization of sequence with at least about 85% sequence identity, preferably with at least about 90% sequence identity, more preferably with at least about 95% sequence identity. Hybridization conditions and probes can be adjusted in well-characterized ways to achieve selective hybridization of human-derived probes. Stringent conditions, within the meaning of the invention are 65°C in a buffer containing 1 mM EDTA, 0.5 M NaHPO₄ (pH 7.2), 7 % (w/v) SDS.

Nucleic acid molecules that will hybridize to PRKG1 polynucleotides under stringent conditions can be identified functionally. Without limitation, examples of the uses for hybridization probes include: histochemical uses such as identifying tissues that express PRKG1; measuring mRNA

levels, for instance to identify a sample's tissue type or to identify cells that express abnormal levels of PRKG1; and detecting polymorphisms of PRKG1.

PCR provides additional uses for oligonucleotides based upon the nucleotide sequence which encodes PRKG1. Such probes used in PCR may be of recombinant origin, chemically synthesized, 5 or a mixture of both. Oligomers may comprise discrete nucleotide sequences employed under optimized conditions for identification of PRKG1 in specific tissues or diagnostic use. The same two oligomers, a nested set of oligomers, or even a degenerate pool of oligomers may be employed under less stringent conditions for identification of closely related DNAs or RNAs.

Rules for designing polymerase chain reaction (PCR) primers are now established, as reviewed by 10 PCR Protocols. Degenerate primers, i.e., preparations of primers that are heterogeneous at given sequence locations, can be designed to amplify nucleic acid sequences that are highly homologous to, but not identical with PRKG1. Strategies are now available that allow for only one of the primers to be required to specifically hybridize with a known sequence. For example, appropriate nucleic acid primers can be ligated to the nucleic acid sought to be amplified to provide the 15 hybridization partner for one of the primers. In this way, only one of the primers need be based on the sequence of the nucleic acid sought to be amplified.

PCR methods for amplifying nucleic acid will utilize at least two primers. One of these primers will be capable of hybridizing to a first strand of the nucleic acid to be amplified and of priming enzyme-driven nucleic acid synthesis in a first direction. The other will be capable of hybridizing 20 the reciprocal sequence of the first strand (if the sequence to be amplified is single stranded, this sequence will initially be hypothetical, but will be synthesized in the first amplification cycle) and of priming nucleic acid synthesis from that strand in the direction opposite the first direction and towards the site of hybridization for the first primer. Conditions for conducting such amplifications, particularly under preferred stringent hybridization conditions, are well known.

25 Other means of producing specific hybridization probes for PRKG1 include the cloning of nucleic acid sequences encoding PRKG1 or PRKG1 derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerase as T7 or SP6 RNA polymerase and the appropriate reporter molecules.

30 It is possible to produce a DNA sequence, or portions thereof, entirely by synthetic chemistry. After synthesis, the nucleic acid sequence can be inserted into any of the many available DNA vectors and their respective host cells using techniques which are well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into the nucleotide sequence.

Alternately, a portion of sequence in which a mutation is desired can be synthesized and recombined with longer portion of an existing genomic or recombinant sequence.

PRKG1 polynucleotides may be used to produce a purified oligo- or polypeptide using well known methods of recombinant DNA technology. The oligopeptide may be expressed in a variety of host 5 cells, either prokaryotic or eukaryotic. Host cells may be from the same species from which the nucleotide sequence was derived or from a different species. Advantages of producing an oligonucleotide by recombinant DNA technology include obtaining adequate amounts of the protein for purification and the availability of simplified purification procedures.

Quantitative determinations of nucleic acids

10 An important step in the molecular genetic analysis of human disease is often the enumeration of the copy number of a nucleic acid or the relative expression of a gene in particular tissues.

Several different approaches are currently available to make quantitative determinations of nucleic acids. Chromosome-based techniques, such as comparative genomic hybridization (CGH) and fluorescent in situ hybridization (FISH) facilitate efforts to cytogenetically localize genomic 15 regions that are altered in tumor cells. Regions of genomic alteration can be narrowed further using loss of heterozygosity analysis (LOH), in which disease DNA is analyzed and compared with normal DNA for the loss of a heterozygous polymorphic marker. The first experiments used restriction fragment length polymorphisms (RFLPs) [Johnson, (1989)], or hypervariable minisatellite DNA [Barnes, 2000]. In recent years LOH has been performed primarily using PCR 20 amplification of microsatellite markers and electrophoresis of the radio labelled [Jeffreys, (1985)] or fluorescently labelled PCR products [Weber, (1990)] and compared between paired normal and disease DNAs.

A number of other methods have also been developed to quantify nucleic acids [Gergen, (1992)]. More recently, PCR and RT-PCR methods have been developed which are capable of measuring 25 the amount of a nucleic acid in a sample. One approach, for example, measures PCR product quantity in the log phase of the reaction before the formation of reaction products plateaus [Thomas, (1980)].

A gene sequence contained in all samples at relatively constant quantity is typically utilized for sample amplification efficiency normalization. This approach, however, suffers from several 30 drawbacks. The method requires that each sample has equal input amounts of the nucleic acid and that the amplification efficiency between samples is identical until the time of analysis. Furthermore, it is difficult using the conventional methods of PCR quantitation such as gel

electrophoresis or plate capture hybridization to determine that all samples are in fact analyzed during the log phase of the reaction as required by the method.

Another method called quantitative competitive (QC)-PCR, as the name implies, relies on the inclusion of an internal control competitor in each reaction [Piatak, (1993), BioTechniques]. The 5 efficiency of each reaction is normalized to the internal competitor. A known amount of internal competitor is typically added to each sample. The unknown target PCR product is compared with the known competitor PCR product to obtain relative quantitation. A difficulty with this general approach lies in developing an internal control that amplifies with the same efficiency than the target molecule.

10 *5' Fluorogenic Nuclease Assays*

Fluorogenic nuclease assays are a real time quantitation method that uses a probe to monitor formation of amplification product. The basis for this method of monitoring the formation of amplification product is to measure continuously PCR product accumulation using a dual-labelled fluorogenic oligonucleotide probe, an approach frequently referred to in the literature simply as the 15 "TaqMan method" [Piatak, (1993), Science; Heid, (1996); Gibson, (1996); Holland. (1991)].

The probe used in such assays is typically a short (about 20-25 bases) oligonucleotide that is labeled with two different fluorescent dyes. The 5' terminus of the probe is attached to a reporter dye and the 3' terminus is attached to a quenching dye, although the dyes could be attached at other locations on the probe as well. The probe is designed to have at least substantial sequence 20 complementarity with the probe binding site. Upstream and downstream PCR primers which bind to flanking regions of the locus are added to the reaction mixture. When the probe is intact, energy transfer between the two fluorophors occurs and the quencher quenches emission from the reporter. During the extension phase of PCR, the probe is cleaved by the 5' nuclease activity of a nucleic acid polymerase such as Taq polymerase, thereby releasing the reporter from the 25 oligonucleotide-quencher and resulting in an increase of reporter emission intensity which can be measured by an appropriate detector.

One detector which is specifically adapted for measuring fluorescence emissions such as those created during a fluorogenic assay is the ABI 7700 or 4700 HT manufactured by Applied Biosystems, Inc. in Foster City, Calif. The ABI 7700 uses fiber optics connected with each well in 30 a 96-or 384 well PCR tube arrangement. The instrument includes a laser for exciting the labels and is capable of measuring the fluorescence spectra intensity from each tube with continuous monitoring during PCR amplification. Each tube is re-examined every 8.5 seconds.

Computer software provided with the instrument is capable of recording the fluorescence intensity of reporter and quencher over the course of the amplification. The recorded values will then be used to calculate the increase in normalized reporter emission intensity on a continuous basis. The increase in emission intensity is plotted versus time, i.e., the number of amplification cycles, to produce a continuous measure of amplification. To quantify the locus in each amplification reaction, the amplification plot is examined at a point during the log phase of product accumulation. This is accomplished by assigning a fluorescence threshold intensity above background and determining the point at which each amplification plot crosses the threshold (defined as the threshold cycle number or Ct). Differences in threshold cycle number are used to quantify the relative amount of PCR target contained within each tube. Assuming that each reaction functions at 100% PCR efficiency, a difference of one Ct represents a two-fold difference in the amount of starting template. The fluorescence value can be used in conjunction with a standard curve to determine the amount of amplification product present.

Non-Probe-Based Detection Methods

- 15 A variety of options are available for measuring the amplification products as they are formed. One method utilizes labels, such as dyes, which only bind to double stranded DNA. In this type of approach, amplification product (which is double stranded) binds dye molecules in solution to form a complex. With the appropriate dyes, it is possible to distinguish between dye molecules free in solution and dye molecules bound to amplification product. For example, certain dyes fluoresce only when bound to amplification product. Examples of dyes which can be used in methods of this general type include, but are not limited to, Syber Green.TM. and Pico Green from Molecular Probes, Inc. of Eugene, Oreg., ethidium bromide, propidium iodide, chromomycin, acridine orange, Hoechst 33258, Toto-1, Yoyo-1, DAPI (4',6-diamidino-2-phenylindole hydrochloride).
- 20 25 Another real time detection technique measures alteration in energy fluorescence energy transfer between fluorophors conjugated with PCR primers [Livak, (1995)].

Probe-Based Detection Methods

- These detection methods involve some alteration to the structure or conformation of a probe hybridized to the locus between the amplification primer pair. In some instances, the alteration is caused by the template-dependent extension catalyzed by a nucleic acid polymerase during the amplification process. The alteration generates a detectable signal which is an indirect measure of the amount of amplification product formed.

For example, some methods involve the degradation or digestion of the probe during the extension reaction. These methods are a consequence of the 5'-3' nuclease activity associated with some nucleic acid polymerases. Polymerases having this activity cleave mononucleotides or small oligonucleotides from an oligonucleotide probe annealed to its complementary sequence located 5 within the locus.

The 3' end of the upstream primer provides the initial binding site for the nucleic acid polymerase. As the polymerase catalyzes extension of the upstream primer and encounters the bound probe, the nucleic acid polymerase displaces a portion of the 5' end of the probe and through its nuclease activity cleaves mononucleotides or oligonucleotides from the probe.

- 10 The upstream primer and the probe can be designed such that they anneal to the complementary strand in close proximity to one another. In fact, the 3' end of the upstream primer and the 5' end of the probe may abut one another. In this situation, extension of the upstream primer is not necessary in order for the nucleic acid polymerase to begin cleaving the probe. In the case in which intervening nucleotides separate the upstream primer and the probe, extension of the primer 15 is necessary before the nucleic acid polymerase encounters the 5' end of the probe. Once contact occurs and polymerization continues, the 5'-3' exonuclease activity of the nucleic acid polymerase begins cleaving mononucleotides or oligonucleotides from the 5' end of the probe. Digestion of the probe continues until the remaining portion of the probe dissociates from the complementary strand.
- 20 In solution, the two end sections can hybridize with each other to form a hairpin loop. In this conformation, the reporter and quencher dye are in sufficiently close proximity that fluorescence from the reporter dye is effectively quenched by the quencher dye. Hybridized probe, in contrast, results in a linearized conformation in which the extent of quenching is decreased. Thus, by monitoring emission changes for the two dyes, it is possible to indirectly monitor the formation of 25 amplification product.

Probes

The labeled probe is selected so that its sequence is substantially complementary to a segment of the test locus or a reference locus. As indicated above, the nucleic acid site to which the probe binds should be located between the primer binding sites for the upstream and downstream

30 amplification primers.

Primers

The primers used in the amplification are selected so as to be capable of hybridizing to sequences at flanking regions of the locus being amplified. The primers are chosen to have at least substantial complementarity with the different strands of the nucleic acid being amplified. When a 5 probe is utilized to detect the formation of amplification products, the primers are selected in such that they flank the probe, i.e. are located upstream and downstream of the probe.

The primer must have sufficient length so that it is capable of priming the synthesis of extension products in the presence of an agent for polymerization. The length and composition of the primer depends on many parameters, including, for example, the temperature at which the annealing 10 reaction is conducted, proximity of the probe binding site to that of the primer, relative concentrations of the primer and probe and the particular nucleic acid composition of the probe. Typically the primer includes 15-30 nucleotides. However, the length of the primer may be more or less depending on the complexity of the primer binding site and the factors listed above.

Labels for Probes and Primers

15 The labels used for labeling the probes or primers of the current invention and which can provide the signal corresponding to the quantity of amplification product can take a variety of forms. As indicated above with regard to the 5' fluorogenic nuclease method, a fluorescent signal is one signal which can be measured. However, measurements may also be made, for example, by monitoring radioactivity, colorimetry, absorption, magnetic parameters, or enzymatic activity. 20 Thus, labels which can be employed include, but are not limited to, fluorophors, chromophores, radioactive isotopes, electron dense reagents, enzymes, and ligands having specific binding partners (e.g., biotin-avidin).

Monitoring changes in fluorescence is a particularly useful way to monitor the accumulation of 25 amplification products. A number of labels useful for attachment to probes or primers are commercially available including fluorescein and various fluorescein derivatives such as FAM, HEX, TET and JOE (all which are available from Applied Biosystems, Foster City, Calif.); lucifer yellow, and coumarin derivatives.

Labels may be attached to the probe or primer using a variety of techniques and can be attached at the 5' end, and/or the 3' end and/or at an internal nucleotide. The label can also be attached to 30 spacer arms of various sizes which are attached to the probe or primer. These spacer arms are useful for obtaining a desired distance between multiple labels attached to the probe or primer.

In some instances, a single label may be utilized; whereas, in other instances, such as with the 5' fluorogenic nuclease assays for example, two or more labels are attached to the probe. In cases wherein the probe includes multiple labels, it is generally advisable to maintain spacing between the labels which is sufficient to permit separation of the labels during digestion of the probe
5 through the 5'-3' nuclease activity of the nucleic acid polymerase.

Patients Exhibiting Symptoms of Disease

A number of diseases are associated with changes in the copy number of a certain gene. For patients having symptoms of a disease, the real-time PCR method can be used to determine if the patient has copy number alterations which are known to be linked with diseases that are associated
10 with the symptoms the patient has.

PRKG1 expression

PRKG1 fusion proteins

Fusion proteins are useful for generating antibodies against PRKG1 polypeptides and for use in various assay systems. For example, fusion proteins can be used to identify proteins which
15 interact with portions of PRKG1 polypeptides. Protein affinity chromatography or library-based assays for protein-protein interactions, such as the yeast two-hybrid or phage display systems, can be used for this purpose. Such methods are well known in the art and also can be used as drug screens.

A PRKG1 fusion protein comprises two polypeptide segments fused together by means of a
20 peptide bond. The first polypeptide segment can comprise at least 54, 75, 100, 125, 139, 150, 175, 200, 225, 250, 275, 300, 325 or 350 contiguous amino acids of SEQ ID NO: 2 or of a biologically active variant, such as those described above. The first polypeptide segment also can comprise full-length PRKG1.

The second polypeptide segment can be a full-length protein or a protein fragment. Proteins
25 commonly used in fusion protein construction include, but are not limited to β galactosidase, β -glucuronidase, green fluorescent protein (GFP), autofluorescent proteins, including blue fluorescent protein (BFP), glutathione-S-transferase (GST), luciferase, horseradish peroxidase (HRP), and chloramphenicol acetyltransferase (CAT). Additionally, epitope tags are used in fusion protein constructions, including histidine (His) tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Other fusion constructions can include maltose binding protein (MBP), S-tag, Lex a DNA binding domain (DBD) fusions, GAL4

DNA binding domain fusions, and herpes simplex virus (HSV) BP16 protein fusions. A fusion protein also can be engineered to contain a cleavage site located adjacent to the PRKG1.

Preparation of Polynucleotides

A naturally occurring PRKG1 polynucleotide can be isolated free of other cellular components such as membrane components, proteins, and lipids. Polynucleotides can be made by a cell and isolated using standard nucleic acid purification techniques, or synthesized using an amplification technique, such as the polymerase chain reaction (PCR), or by using an automatic synthesizer. Methods for isolating polynucleotides are routine and are known in the art. Any such technique for obtaining a polynucleotide can be used to obtain isolated PRKG1 polynucleotides. For example, restriction enzymes and probes can be used to isolate polynucleotide fragments which comprise PRKG1 nucleotide sequences. Isolated polynucleotides are in preparations which are free or at least 70, 80, or 90% free of other molecules.

PRKG1 cDNA molecules can be made with standard molecular biology techniques, using PRKG1 mRNA as a template. PRKG1 cDNA molecules can thereafter be replicated using molecular biology techniques known in the art. An amplification technique, such as PCR, can be used to obtain additional copies of polynucleotides of the invention, using either human genomic DNA or cDNA as a template.

Alternatively, synthetic chemistry techniques can be used to synthesize PRKG1 polynucleotides. The degeneracy of the genetic code allows alternate nucleotide sequences to be synthesized which will encode PRKG1 having, for example, an amino acid sequence shown in SEQ ID NO: 2 or a biologically active variant thereof.

Extending Polynucleotides

Various PCR-based methods can be used to extend nucleic acid sequences encoding human PRKG1, for example to detect upstream sequences of PRKG1 gene such as promoters and regulatory elements. For example, restriction-site PCR uses universal primers to retrieve unknown sequence adjacent to a known locus. Genomic DNA is first amplified in the presence of a primer to a linker sequence and a primer specific to the known region. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR also can be used to amplify or extend sequences using divergent primers based on a known region. Primers can be designed using commercially available software, such as OLIGO

4.06 Primer Analysis software (National Biosciences Inc., Plymouth, Minn.), to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68-72°C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular
5 ligation and used as a PCR template.

Another method which can be used is capture PCR, which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA. In this method, multiple restriction enzyme digestions and ligations also can be used to place an engineered double-stranded sequence into an unknown fragment of the DNA molecule before
10 performing PCR.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Randomly-primed libraries are preferable, in that they will contain more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length
15 cDNA. Genomic libraries can be useful for extension of sequence into 5' non-transcribed regulatory regions.

Commercially available capillary electrophoresis systems can be used to analyze the size or confirm the nucleotide sequence of PCR or sequencing products. For example, capillary sequencing can employ flowable polymers for electrophoretic separation, four different fluorescent
20 dyes (one for each nucleotide) which are laser activated, and detection of the emitted wavelengths by a charge coupled device camera. Output/light intensity can be converted to electrical signal using appropriate equipment and software (*e.g.*, GENOTYPER and Sequence NAVIGATOR, Perkin Elmer), and the entire process from loading of samples to computer analysis and electronic data display can be computer controlled. Capillary electrophoresis is especially preferable for the
25 sequencing of small pieces of DNA which might be present in limited amounts in a particular sample.

Obtaining Polypeptides

PRKG1 can be obtained, for example, by purification from human cells, by expression of PRKG1 polynucleotides, or by direct chemical synthesis.

30 Protein Purification

PRKG1 can be purified from any human cell which expresses the enzyme, including those which have been transfected with expression constructs which express PRKG1. A purified PRKG1 is

separated from other compounds which normally associate with PRKG1 in the cell, such as certain proteins, carbohydrates, or lipids, using methods well-known in the art. Such methods include, but are not limited to, size exclusion chromatography, ammonium sulfate fractionation, ion exchange chromatography, affinity chromatography, and preparative gel electrophoresis.

5 *Expression of PRKG1 Polynucleotides*

To express PRKG1, PRKG1 polynucleotides can be inserted into an expression vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art can be used to construct expression vectors containing sequences encoding PRKG1 and appropriate transcriptional and 10 translational control elements. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination.

A variety of expression vector/host systems can be utilized to contain and express sequences encoding PRKG1. These include, but are not limited to, microorganisms, such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast 15 transformed with yeast expression vectors, insect cell systems infected with virus expression vectors (*e.g.*, baculovirus), plant cell systems transformed with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmids), or animal cell systems.

The control elements or regulatory sequences are those non-translated regions of the vector - 20 enhancers, promoters, 5' and 3' untranslated regions -- which interact with host cellular proteins to carry out transcription and translation. Such elements can vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, can be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the 25 BLUESCRIPT phagemid (Stratagene, LaJolla, Calif.) or pSPORT1 plasmid (Life Technologies) and the like can be used. The baculovirus polyhedrin promoter can be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (*e.g.*, heat shock, RUBISCO, and storage protein genes) or from plant viruses (*e.g.*, viral promoters or leader sequences) can be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from 30 mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of a nucleotide sequence encoding PRKG1, vectors based on SV40 or EBV can be used with an appropriate selectable marker.

Bacterial and Yeast Expression Systems

In bacterial systems, a number of expression vectors can be selected. For example, when a large quantity of PRKG1 is needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified can be used. Such vectors include, but are

5 not limited to, multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene). In a BLUESCRIPT vector, a sequence encoding PRKG1 can be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β -galactosidase so that a hybrid protein is produced. pIN vectors or pGEX vectors (Promega, Madison, Wis.) also can be used to express foreign polypeptides as fusion proteins with
10 glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems can be designed to include heparin, thrombin, or factor Xa protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

15 *Plant and Insect Expression Systems*

If plant expression vectors are used, the expression of sequences encoding PRKG1 can be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV can be used alone or in combination with the omega leader sequence from TMV. Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock
20 promoters can be used. These constructs can be introduced into plant cells by direct DNA transformation or by pathogen-mediated transfection.

An insect system also can be used to express PRKG1. For example, in one such system *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. Sequences encoding PRKG1 can
25 be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of PRKG1 will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses can then be used to infect *S. frugiperda* cells or *Trichoplusia* larvae in which PRKG1 can be expressed.

30 *Mammalian Expression Systems*

A number of viral-based expression systems can be used to express PRKG1 in mammalian host cells. For example, if an adenovirus is used as an expression vector, sequences encoding PRKG1

can be ligated into an adenovirus transcription/translation complex comprising the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome can be used to obtain a viable virus which is capable of expressing PRKG1 in infected host cells [Engelhard, 1994]. If desired, transcription enhancers, such as the Rous sarcoma virus (RSV) 5 enhancer, can be used to increase expression in mammalian host cells.

Human artificial chromosomes (HACs) also can be used to deliver larger fragments of DNA than can be contained and expressed in a plasmid. HACs of 6M to 10M are constructed and delivered to cells via conventional delivery methods (*e.g.*, liposomes, polycationic amino polymers, or vesicles). Specific initiation signals also can be used to achieve more efficient translation of 10 sequences encoding PRKG1. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding PRKG1, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals (including the ATG initiation 15 codon) should be provided. The initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons can be of various origins, both natural and synthetic.

Host Cells

A host cell strain can be chosen for its ability to modulate the expression of the inserted sequences 20 or to process the expressed PRKG1 in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a “pro” form of the polypeptide also can be used to facilitate correct insertion, folding and/or function. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational 25 activities (*e.g.*, CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC; 10801 University Boulevard, Manassas, VA 20110-2209) and can be chosen to ensure the correct modification and processing of the foreign protein.

Stable expression is preferred for long-term, high-yield production of recombinant proteins. For example, cell lines which stably express PRKG1 can be transformed using expression vectors 30 which can contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells can be allowed to grow for 1-2 days in an enriched medium before they are switched to a selective medium. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced

PRKG1 sequences. Resistant clones of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type. Any number of selection systems can be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase [Logan, (1984)] and adenine phosphoribosyltransferase [Wigler, (1977)] genes which can be employed in *tk* or *aprt* cells, respectively. Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate [Lowy, (1980)], *npt* confers resistance to the aminoglycosides, neomycin and G-418 [Wigler, (1980)], and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively [Colbere-Garapin, 1981]. Additional selectable genes have been described. For example, *trpB* allows cells to utilize indole in place of tryptophan, or *hisD*, which allows cells to utilize histinol in place of histidine. Visible markers such as anthocyanins, β -glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, can be used to identify transformants and to quantify the amount of transient or stable protein expression attributable to a specific vector system

15 *Detecting Polypeptide Expression*

Although the presence of marker gene expression suggests that a PRKG1 polynucleotide is also present, its presence and expression may need to be confirmed. For example, if a sequence encoding PRKG1 is inserted within a marker gene sequence, transformed cells containing sequences which encode PRKG1 can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding PRKG1 under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of PRKG1 polynucleotide.

Alternatively, host cells which contain a PRKG1 polynucleotide and which express PRKG1 can be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immuno-assay techniques which include membrane, solution, or chip-based technologies for the detection and/or quantification of nucleic acid or protein. For example, the presence of a polynucleotide sequence encoding PRKG1 can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or fragments or fragments of polynucleotides encoding PRKG1. Nucleic acid amplification-based assays involve the use of oligonucleotides selected from sequences encoding PRKG1 to detect transformants which contain a PRKG1 polynucleotide.

A variety of protocols for detecting and measuring the expression of PRKG1, using either polyclonal or monoclonal antibodies specific for the polypeptide, are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence

activated cell sorting (FACS). A two-site, monoclonal-based immunoassay using monoclonal antibodies reactive to two non-interfering epitopes on PRKG1 can be used, or a competitive binding assay can be employed.

A wide variety of labels and conjugation techniques are known by those skilled in the art and can be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding PRKG1 include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, sequences encoding PRKG1 can be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and can be used to synthesize RNA probes *in vitro* by addition of labeled nucleotides and an appropriate RNA polymerase such as T7, T3, or SP6. These procedures can be conducted using a variety of commercially available kits (Amersham Pharmacia Biotech, Promega, and US Biochemical). Suitable reporter molecules or labels which can be used for ease of detection include radionuclides, enzymes, and fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Expression and Purification of Polypeptides

Host cells transformed with PRKG1 polynucleotides can be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The polypeptide produced by a transformed cell can be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing PRKG1 polynucleotides can be designed to contain signal sequences which direct secretion of soluble PRKG1 through a prokaryotic or eukaryotic cell membrane or which direct the membrane insertion of membrane-bound PRKG1.

As discussed above, other constructions can be used to join a sequence encoding PRKG1 to a nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). Inclusion of cleavable linker sequences such as those specific for Factor XA or enterokinase (Invitrogen, San Diego, CA) between the purification domain and PRKG1 also can be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing PRKG1 and 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification by IMAC (immobilized metal ion affinity

chromatography) Maddox, (1983)], while the enterokinase cleavage site provides a means for purifying PRKG1 from the fusion protein [Porath, (1992)].

Chemical Synthesis

Sequences encoding PRKG1 can be synthesized, in whole or in part, using chemical methods well known in the art. Alternatively, PRKG1 itself can be produced using chemical methods to synthesize its amino acid sequence, such as by direct peptide synthesis using solid-phase techniques. Protein synthesis can either be performed using manual techniques or by automation. Automated synthesis can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Optionally, fragments of PRKG1 can be separately synthesized and combined using chemical methods to produce a full-length molecule.

The newly synthesized peptide can be substantially purified by preparative high performance liquid chromatography. The composition of a synthetic PRKG1 can be confirmed by amino acid analysis or sequencing. Additionally, any portion of the amino acid sequence of PRKG1 can be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins to produce a variant polypeptide or a fusion protein.

Production of Altered Polypeptides

As will be understood by those of skill in the art, it may be advantageous to produce PRKG1 polynucleotides possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce an RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

The nucleotide sequences referred to herein can be engineered using methods generally known in the art to alter PRKG1 polynucleotides for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the polypeptide or mRNA product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides can be used to engineer the nucleotide sequences. For example, site-directed mutagenesis can be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, introduce mutations, and so forth.

PRKG1 Analogs

One general class of PRKG1 analogs are variants having an amino acid sequence that is a mutation of the amino acid sequence disclosed herein. Another general class of PRKG1 analogs is provided

by anti-idiotype antibodies, and fragments thereof, as described below. Moreover, recombinant antibodies comprising anti-idiotype variable domains can be used as analogs (see, for example, [Monfardini et al., (1996)]). Since the variable domains of anti-idiotype PRKG1 antibodies mimic PRKG1, these domains can provide PRKG1 enzymatic activity. Methods of producing anti-
5 idiotypic catalytic antibodies are known to those of skill in the art [Joron et al., (1992), Friboulet et al. (1994), Avalle et al., (1998)].

Another approach to identifying PRKG1 analogs is provided by the use of combinatorial libraries. Methods for constructing and screening phage display and other combinatorial libraries are provided, for example, by [Kay et al., Phage Display of Peptides and Proteins (Academic Press 10 1996), U.S. 5,783,384, U.S. 5,747,334, and U.S. 5,723,323.

Antibodies

Any type of antibody known in the art can be generated to bind specifically to an epitope of PRKG1.

“Antibody” as used herein includes intact immunoglobulin molecules, as well as fragments thereof, such as Fab, F(ab')₂, and Fv, which are capable of binding an epitope of PRKG1. Typically, at least 6, 8, 10, or 12 contiguous amino acids are required to form an epitope. However, epitopes which involve non-contiguous amino acids may require more, e.g., at least 15, 20 25, or 50 amino acid. An antibody which specifically binds to an epitope of PRKG1 can be used therapeutically, as well as in immunochemical assays, such as Western blots, ELISAs, radioimmunoassays, immunohistochemical assays, immunoprecipitations, or other immunochemical assays known in the art. Various immunoassays can be used to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays are well known in the art. Such immunoassays typically involve the measurement of complex formation between an immunogen and an antibody which specifically binds to the PRKG1 25 immunogen.

Typically, an antibody which specifically binds to PRKG1 provides a detection signal at least 5-, 10-, or 20-fold higher than a detection signal provided with other proteins when used in an immunochemical assay. Preferably, antibodies which specifically bind to PRKG1 do not detect other proteins in immunochemical assays and can immunoprecipitate PRKG1 from solution.

PRKG1 can be used to immunize a mammal, such as a mouse, rat, rabbit, guinea pig, monkey, or 30 human, to produce polyclonal antibodies. If desired, PRKG1 can be conjugated to a carrier protein, such as bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin. Depending

on the host species, various adjuvants can be used to increase the immunological response. Such adjuvants include, but are not limited to, Freund's adjuvant, mineral gels (e.g., aluminum hydroxide), and surface active substances (e.g., lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol). Among adjuvants used in
5 humans, BCG (*bacilli Calmette-Guerin*) and *Corynebacterium parvum* are especially useful.

Monoclonal antibodies which specifically bind to PRKG1 can be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These techniques include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique [Roberge, (1995)].

- 10 In addition, techniques developed for the production of "chimeric antibodies", the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used. Monoclonal and other antibodies also can be "humanized" to prevent a patient from mounting an immune response against the antibody when it is used therapeutically. Such antibodies may be sufficiently similar in sequence to human
15 antibodies to be used directly in therapy or may require alteration of a few key residues. Sequence differences between rodent antibodies and human sequences can be minimized by replacing residues which differ from those in the human sequences by site directed mutagenesis of individual residues or by grafting of entire complementarity determining regions. Antibodies which specifically bind to PRKG1 can contain antigen binding sites which are either partially or fully
20 humanized, as disclosed in U.S. 5,565,332.

Alternatively, techniques described for the production of single chain antibodies can be adapted using methods known in the art to produce single chain antibodies which specifically bind to PRKG1. Antibodies with related specificity, but of distinct idiotypic composition, can be generated by chain shuffling from random combinatorial immunoglobulin libraries. Single-chain
25 antibodies also can be constructed using a DNA amplification method, such as PCR, using hybridoma cDNA as a template. Single-chain antibodies can be mono- or bispecific, and can be bivalent or tetravalent. Construction of tetravalent, bispecific single-chain antibodies is taught. A nucleotide sequence encoding a single-chain antibody can be constructed using manual or automated nucleotide synthesis, cloned into an expression construct using standard recombinant DNA methods, and introduced into a cell to express the coding sequence, as described below.
30 Alternatively, single-chain antibodies can be produced directly using, for example, filamentous phage technology.

Antibodies which specifically bind to PRKG1 also can be produced by inducing *in vivo* production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific

binding reagents. Other types of antibodies can be constructed and used therapeutically in methods of the invention. For example, chimeric antibodies can be constructed as disclosed in WO 93/03151. Binding proteins which are derived from immunoglobulins and which are multivalent and multispecific, such as the "diabodies" described in WO 94/13804, also can be 5 prepared.

Antibodies according to the invention can be purified by methods well known in the art. For example, antibodies can be affinity purified by passage over a column to which PRKG1 is bound. The bound antibodies can then be eluted from the column using a buffer with a high salt concentration.

10 *Antisense Oligonucleotides*

Antisense oligonucleotides are nucleotide sequences which are complementary to a specific DNA or RNA sequence. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form complexes and block either transcription or translation. Preferably, an antisense oligonucleotide is at least 11 nucleotides in length, but can be 15 at least 12, 15, 20, 25, 30, 35, 40, 45, or 50 or more nucleotides long. Longer sequences also can be used. Antisense oligonucleotide molecules can be provided in a DNA construct and introduced into a cell as described above to decrease the level of PRKG1 gene products in the cell.

Antisense oligonucleotides can be deoxyribonucleotides, ribonucleotides, or a combination of both. Oligonucleotides can be synthesized manually or by an automated synthesizer, by covalently 20 linking the 5' end of one nucleotide with the 3' end of another nucleotide with non-phosphodiester internucleotide linkages such as alkylphosphonates, phosphorothioates, phosphorodithioates, alkyl-phosphonothioates, alkylphosphonates, phosphoramidates, phosphate esters, carbamates, acetamide, carboxymethyl esters, carbonates, and phosphate triesters.

Modifications of PRKG1 gene expression can be obtained by designing antisense oligonucleotides 25 which will form duplexes to the control, 5', or regulatory regions of the PRKG1 gene. Oligonucleotides derived from the transcription initiation site, e.g., between positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or 30 chaperons. Therapeutic advances using triplex DNA have been described in the literature [Nicholls, (1993)]. An antisense oligonucleotide also can be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Precise complementarity is not required for successful complex formation between an antisense oligonucleotide and the complementary sequence of a PRKG1 polynucleotide. Antisense oligonucleotides which comprise, for example, 2, 3, 4, or 5 or more stretches of contiguous nucleotides which are precisely complementary to a PRKG1 polynucleotide, each separated by a
5 stretch of contiguous nucleotides which are not complementary to adjacent PRKG1 nucleotides, can provide sufficient targeting specificity for PRKG1 mRNA. Preferably, each stretch of complementary contiguous nucleotides is at least 4, 5, 6, 7, or 8 or more nucleotides in length. Non-complementary intervening sequences are preferably 1, 2, 3, or 4 nucleotides in length. One skilled in the art can easily use the calculated melting point of an antisense-sense pair to determine
10 the degree of mismatching which will be tolerated between a particular antisense oligonucleotide and a particular PRKG1 polynucleotide sequence. Antisense oligonucleotides can be modified without affecting their ability to hybridize to a PRKG1 polynucleotide. These modifications can be internal or at one or both ends of the antisense molecule. For example, internucleoside phosphate linkages can be modified by adding cholesteryl or diamine moieties with varying
15 numbers of carbon residues between the amino groups and terminal ribose. Modified bases and/or sugars, such as arabinose instead of ribose, or a 3', 5'-substituted oligonucleotide in which the 3' hydroxyl group or the 5' phosphate group are substituted, also can be employed in a modified antisense oligonucleotide. These modified oligonucleotides can be prepared by methods well known in the art.

20 *Ribozymes*

Ribozymes are RNA molecules with catalytic activity [Uhlmann, (1987)]. Ribozymes can be used to inhibit gene function by cleaving an RNA sequence, as is known in the art. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples include engineered
25 hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of specific nucleotide sequences. The coding sequence of a PRKG1 polynucleotide can be used to generate ribozymes which will specifically bind to mRNA transcribed from a PRKG1 polynucleotide. Methods of designing and constructing ribozymes which can cleave other RNA molecules in trans in a highly sequence specific manner have been developed
30 and described in the art. For example, the cleavage activity of ribozymes can be targeted to specific RNAs by engineering a discrete "hybridization" region into the ribozyme. The hybridization region contains a sequence complementary to the target RNA and thus specifically hybridizes with the target RNA.

Specific ribozyme cleavage sites within a PRKG1 RNA target can be identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target RNA containing the cleavage site can be evaluated for secondary structural features which may render the target inoperable. Suitability of candidate PRKG1 RNA targets also can be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays. The nucleotide sequences shown in SEQ ID NO: 1 and its complement provide sources of suitable hybridization region sequences. Longer complementary sequences can be used to increase the affinity of the hybridization sequence for the target. The hybridizing and cleavage regions of the ribozyme can be integrally related such that upon hybridizing to the target RNA through the complementary regions, the catalytic region of the ribozyme can cleave the target.

Ribozymes can be introduced into cells as part of a DNA construct. Mechanical methods, such as microinjection, liposome-mediated transfection, electroporation, or calcium phosphate precipitation, can be used to introduce a ribozyme-containing DNA construct into cells in which it is desired to decrease PRKG1 expression. Alternatively, if it is desired that the cells stably retain the DNA construct, the construct can be supplied on a plasmid and maintained as a separate element or integrated into the genome of the cells, as is known in the art. A ribozyme-encoding DNA construct can include transcriptional regulatory elements, such as a promoter element, an enhancer or UAS element, and a transcriptional terminator signal, for controlling transcription of ribozymes in the cells (U.S. 5,641,673). Ribozymes also can be engineered to provide an additional level of regulation, so that destruction of mRNA occurs only when both a ribozyme and a target gene are induced in the cells.

Screening / Screening Assays

25 Regulators

Regulators as used herein, refer to compounds that affect the activity of PRKG1 in vivo and/or in vitro. Regulators can be agonists and antagonists of PRKG1 polypeptide and can be compounds that exert their effect on the PRKG1 activity via the enzymatic activity, expression, post-translational modifications or by other means. Agonists of PRKG1 are molecules which, when bound to PRKG1, increase or prolong the activity of PRKG1. Agonists of PRKG1 include proteins, nucleic acids, carbohydrates, small molecules, or any other molecule which activate PRKG1. Antagonists of PRKG1 are molecules which, when bound to PRKG1, decrease the amount or the duration of the activity of PRKG1. Antagonists include proteins, nucleic acids,

carbohydrates, antibodies, small molecules, or any other molecule which decrease the activity of PRKG1.

The term "modulate", as it appears herein, refers to a change in the activity of PRKG1 polypeptide. For example, modulation may cause an increase or a decrease in enzymatic activity,

5 binding characteristics, or any other biological, functional, or immunological properties of PRKG1.

As used herein, the terms "specific binding" or "specifically binding" refer to that interaction between a protein or peptide and an agonist, an antibody, or an antagonist. The interaction is dependent upon the presence of a particular structure of the protein recognized by the binding

10 molecule (i.e., the antigenic determinant or epitope). For example, if an antibody is specific for epitope "A" the presence of a polypeptide containing the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

The invention provides methods (also referred to herein as "screening assays") for identifying

15 compounds which can be used for the treatment of diseases related to PRKG1. The methods entail the identification of candidate or test compounds or agents (e.g., peptides, peptidomimetics, small molecules or other molecules) which bind to PRKG1 and/or have a stimulatory or inhibitory effect on the biological activity of PRKG1 or its expression and then determining which of these compounds have an effect on symptoms or diseases related to PRKG1 in an *in vivo* assay.

20 Candidate or test compounds or agents which bind to PRKG1 and/or have a stimulatory or inhibitory effect on the activity or the expression of PRKG1 are identified either in assays that employ cells which express PRKG1 (cell-based assays) or in assays with isolated PRKG1 (cell-free assays). The various assays can employ a variety of variants of PRKG1 (e.g., full-length

25 PRKG1, a biologically active fragment of PRKG1, or a fusion protein which includes all or a portion of PRKG1). Moreover, PRKG1 can be derived from any suitable mammalian species (e.g., human PRKG1, rat PRKG1 or murine PRKG1). The assay can be a binding assay entailing direct or indirect measurement of the binding of a test compound or a known PRKG1 ligand to PRKG1.

The assay can also be an activity assay entailing direct or indirect measurement of the activity of PRKG1. The assay can also be an expression assay entailing direct or indirect measurement of the

30 expression of PRKG1 mRNA or PRKG1 protein. The various screening assays are combined with an *in vivo* assay entailing measuring the effect of the test compound on the symptoms of diseases related to PRKG1.

The present invention includes biochemical, cell free assays that allow the identification of inhibitors and agonists of kinases suitable as lead structures for pharmacological drug development. Such assays involve contacting a form of PRKG1 (e.g., full-length PRKG1, a biologically active fragment of PRKG1, or a fusion protein comprising all or a portion of PRKG1) with a test compound and determining the ability of the test compound to act as an antagonist (preferably) or an agonist of the enzymatic activity of PRKG1.

The activity of PRKG1 molecules of the present invention can be measured using a variety of assays that measure PRKG1 activity. For example, PRKG1 enzyme activity can be assessed by a standard *in vitro* kinase assay.

PRKG1 can be used in substantial and specific assays related to kinases. Such assays involve any of the known kinase functions or activities or properties useful for diagnosis and treatment of kinase-related conditions that are specific for the subfamily of kinases that the one of the present invention belongs to, particularly in cells and tissues that express the kinase.

PRKG1 is also useful in drug screening assays, in cell-based or cell-free systems.

PRKG1 can be used to identify compounds that modulate kinase activity of the protein in its natural state or an altered form that causes a specific disease or pathology associated with the kinase.

The present invention also relates to a method of screening compounds having inhibitory activity of kinase activity of the proteins of the present invention. This screening method consists of two steps. First, PRKG1 is caused to contact a substrate to be phosphorylated by this protein in the presence of a test compound to detect the kinase activity of the protein of the present invention. Second, the kinase activity detected in step (a) is compared with that detected in the absence of the test compound, and a compound that lowers the kinase activity of the protein of the present invention is selected.

The kinase activity of PRKG1 can be detected, for example, by adding ATP having radioactively labeled phosphate to the reaction system containing the protein of the present invention and the substrate and measuring the radioactivity of the phosphate attached to the substrate.

Both, PRKG1 and appropriate variants and fragments can be used in high-throughput screens to assay candidate compounds for the ability to modulate the kinase activity. These compounds can be further screened against a functional kinase to determine the effect of the compound on the kinase activity. Further, these compounds can be tested in animal or invertebrate systems to

determine activity/effectiveness. Compounds can be identified that activate (agonist) or inactivate (antagonist) the kinase to a desired degree.

Further, PRKG1 can be used to screen a compound for the ability to stimulate or inhibit interaction between the kinase protein and a molecule that normally interacts with the kinase protein, e.g. a

5 substrate or a component of the signal pathway that the kinase protein normally interacts (for example, another kinase). Such assays typically include the steps of combining the kinase protein with a candidate compound under conditions that allow the kinase protein, or fragment, to interact with the target molecule, and to detect the formation of a complex between the protein and the target or to detect the biochemical consequence of the interaction with the kinase protein and the
10 target, such as any of the associated effects of signal transduction such as protein phosphorylation, cAMP turnover, and adenylate cyclase activation, etc.

The invention further includes other end point assays to identify compounds that modulate (stimulate or inhibit) PRKG1 kinase activity. The assays typically involve an assay of events in the signal transduction pathway that indicate kinase activity. Thus, the phosphorylation of a substrate,
15 activation of a protein, a change in the expression of genes that are up- or down-regulated in response to the kinase protein dependent signal cascade can be assayed.

Any of the biological or biochemical functions mediated by the kinase can be used as an endpoint assay. These include all of the biochemical or biochemical/biological events described herein, in the references cited herein, incorporated by reference for these endpoint assay targets, and other
20 functions known to those of ordinary skill in the art.

Binding and/or activating compounds can also be screened by using chimeric kinase proteins in which the amino terminal extracellular domain, or parts thereof, the entire transmembrane domain or subregions, such as any of the seven transmembrane segments or any of the intracellular or extracellular loops and the carboxy terminal intracellular domain, or parts thereof, can be replaced

25 by heterologous domains or subregions. For example, a substrate-binding region can be used that interacts with a different substrate than that which is recognized by the native kinase. Accordingly, a different set of signal transduction components is available as an end-point assay for activation. This allows for assays to be performed in other than the specific host cell from which the kinase is derived.

30 PRKG1 is also useful in competition binding assays in methods designed to discover compounds that interact with the kinase (e.g. binding partners and/or ligands). Thus, a compound is exposed to a kinase polypeptide under conditions that allow the compound to bind or to otherwise interact with the polypeptide. Soluble kinase polypeptide is also added to the mixture. If the test compound

interacts with the soluble kinase polypeptide, it decreases the amount of complex formed or activity from the kinase target. This type of assay is particularly useful in cases in which compounds are sought that interact with specific regions of the kinase.

Agents that modulate PRKG1 can be identified using one or more of the above assays, alone or in combination. It is generally preferable to use a cell-based or cell free system first and then confirm activity in an animal or other model system. Such model systems are well known in the art and can readily be employed in this context.

Test compounds used for this screening methods are not particularly limited and are generally low-molecular-weight compounds, proteins (including antibodies), peptides, etc. Test compounds are either artificially synthesized or natural.

As used herein, an antibody is defined in terms consistent with that recognized within the art: they are multi-subunit proteins produced by a mammalian organism in response to an antigen challenge. The antibodies of the present invention include polyclonal antibodies and monoclonal antibodies, as well as fragments of such antibodies, including, but not limited to, Fab or F(ab').sub.2, and Fv fragments.

Many methods are known for generating and/or identifying antibodies to a given target peptide. Several such methods are described by [Harlow, (1989)].

In general, to generate antibodies, an isolated peptide is used as an immunogen and is administered to a mammalian organism, such as a rat, rabbit or mouse. The full-length protein, an antigenic peptide fragment or a fusion protein can be used. Particularly important fragments are those covering functional domains, such as the domains identified in FIG. 2, and domain of sequence homology or divergence amongst the family, such as those that can readily be identified using protein alignment methods and as presented in the Figures.

Antibodies are preferably prepared from regions or discrete fragments of the kinase proteins. Antibodies can be prepared from any region of the peptide as described herein. However, preferred regions will include those involved in function/activity and/or kinase/binding partner interaction.

Modulators of kinase protein activity identified according to these drug screening assays can be used to treat a subject with a disorder mediated by the kinase pathway, by treating cells or tissues that express the kinase.

This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described

herein in an appropriate animal model. For example, an agent identified as described herein (e.g., a kinase-modulating agent, an antisense kinase nucleic acid molecule, a kinase-specific antibody, or a kinase-binding partner) can be used in an animal or other model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as
5 described herein can be used in an animal or other model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

PRKG1 proteins are also useful to provide a target for diagnosing a disease or predisposition to disease mediated by the peptide. Accordingly, the invention provides methods for detecting the
10 presence, or levels of, the protein (or encoding mRNA) in a cell, tissue, or organism.

One agent for detecting a protein in a sample is an antibody capable of selectively binding to protein. A biological sample includes tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject.

The peptides of the present invention also provide targets for diagnosing active protein activity,
15 disease, or predisposition to disease, in a patient having a variant peptide, particularly activities and conditions that are known for other members of the family of proteins to which the present one belongs. Thus, the peptide can be isolated from a biological sample and assayed for the presence of a genetic mutation that results in aberrant peptide. This includes amino acid substitution, deletion, insertion, rearrangement, (as the result of aberrant splicing events), and inappropriate post-
20 translational modification. Analytic methods include altered electrophoretic mobility, altered tryptic peptide digest, altered kinase activity in cell-based or cell-free assay, alteration in substrate or antibody-binding pattern, altered isoelectric point, direct amino acid sequencing, and any other of the known assay techniques useful for detecting mutations in a protein.

The diseases for which detection of kinase genes in a sample could be diagnostic include diseases
25 in which kinase nucleic acid (DNA and/or RNA) is amplified in comparison to normal cells. By "amplification" is meant increased numbers of kinase DNA or RNA in a cell compared with normal cells. In normal cells, kinases are typically found as single copy genes. In selected diseases, the chromosomal location of the kinase genes may be amplified, resulting in multiple copies of the gene, or amplification. Gene amplification can lead to amplification of kinase RNA, or kinase
30 RNA can be amplified in the absence of kinase DNA amplification.

In vitro techniques for detection of peptide include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence using a detection reagent, such as an antibody or protein binding agent. Alternatively, the peptide can be detected in

vivo in a subject by introducing into the subject a labeled anti-peptide antibody or other types of detection agent. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques. Particularly useful are methods that detect the allelic variant of a peptide expressed in a subject and methods which detect fragments of a peptide in a sample.

Solution in vitro assays can be used to identify a PRKG1 substrate or inhibitor. Solid phase systems can also be used to identify a substrate or inhibitor of a PRKG1 polypeptide. For example, a PRKG1 polypeptide or PRKG1 fusion protein can be immobilized onto the surface of a receptor chip of a commercially available biosensor instrument (BIACORE, Biacore AB; Uppsala, Sweden). The use of this instrument is disclosed, for example, by [Karlsson, (1991), and Cunningham and Wells, (1993)].

In brief, a PRKG1 polypeptide or fusion protein is covalently attached, using amine or sulphhydryl chemistry, to dextran fibers that are attached to gold film within a flow cell. A test sample is then passed through the cell. If a PRKG1 substrate or inhibitor is present in the sample, it will bind to the immobilized polypeptide or fusion protein, causing a change in the refractive index of the medium, which is detected as a change in surface plasmon resonance of the gold film. This system allows the determination on- and off-rates, from which binding affinity can be calculated, and assessment of the stoichiometry of binding, as well as the kinetic effects of PRKG1 mutation. This system can also be used to examine antibody-antigen interactions, and the interactions of other complement/anti-complement pairs.

In one embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of PRKG1. Such assays can employ full-length PRKG1, a biologically active fragment of PRKG1, or a fusion protein which includes all or a portion of PRKG1. As described in greater detail below, the test compound can be obtained by any suitable means, e.g., from conventional compound libraries.

Determining the ability of the test compound to modulate the activity of PRKG1 can be accomplished, for example, by determining the ability of PRKG1 to bind to or interact with a target molecule. The target molecule can be a molecule with which PRKG1 binds or interacts with in nature. The target molecule can be a component of a signal transduction pathway which facilitates transduction of an extracellular signal. The target PRKG1 molecule can be, for example, a second intracellular protein which has catalytic activity or a protein which facilitates the association of downstream signaling molecules with PRKG1.

Determining the ability of PRKG1 to bind to or interact with a target molecule can be accomplished by one of the methods described above for determining direct binding. In one embodiment, determining the ability of a polypeptide of the invention to bind to or interact with a target molecule can be accomplished by determining the activity of the target molecule. For example, the

5 activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (e.g., intracellular Ca^{2+} , diacylglycerol, IP_3 , etc.), detecting catalytic/-enzymatic activity of the target on an appropriate substrate, detecting the induction of a reporter gene (e.g., a regulatory element that is responsive to a polypeptide of the invention operably linked to a nucleic acid encoding a detectable marker, e.g., luciferase), or detecting a cellular response.

10 In various embodiments of the above assay methods of the present invention, it may be desirable to immobilize PRKG1 (or a PRKG1 target molecule) to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to PRKG1, or interaction of PRKG1 with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for
15 containing the reactants. Examples of such vessels include microtitre plates, test tubes, and microcentrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase (GST) fusion proteins or glutathione-S-transferase fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical; St. Louis, Mo.) or glutathione derivatized micro-
20 titre plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or PRKG1, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtitre plate wells are washed to remove any unbound components and complex formation is measured either directly or indirectly, for example, as described above. Alternatively,
25 the complexes can be dissociated from the matrix, and the level of binding or activity of PRKG1 can be determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either PRKG1 or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated polypeptide of the invention or target
30 molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (e.g., biotinylation kit, Pierce Chemicals; Rockford, Ill.), and immobilized in the wells of streptavidin-coated plates (Pierce Chemical). Alternatively, antibodies reactive with PRKG1 or target molecules but which do not interfere with binding of the polypeptide of the invention to its target molecule can be derivatized to the wells of the plate, and unbound target or
35 polypeptide of the invention trapped in the wells by antibody conjugation. Methods for detecting

such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with PRKG1 or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with PRKG1 or target molecule.

- 5 Another technique for drug screening which may be used provides for high throughput screening of compounds having suitable binding affinity to the protein of interest as described in published PCT application WO84/03564. In this method, large numbers of different small test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The test compounds are reacted with PRKG1, or fragments thereof, and washed. Bound PRKG1 is then
10 detected by methods well known in the art. Purified PRKG1 can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding PRKG1 specifically compete with a test compound for binding
15 PRKG1. In this manner, antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with PRKG1.

- The screening assay can also involve monitoring the expression of PRKG1. For example, regulators of expression of PRKG1 can be identified in a method in which a cell is contacted with a candidate compound and the expression of PRKG1 protein or mRNA in the cell is determined.
20 The level of expression of PRKG1 protein or mRNA the presence of the candidate compound is compared to the level of expression of PRKG1 protein or mRNA in the absence of the candidate compound. The candidate compound can then be identified as a regulator of expression of PRKG1 based on this comparison. For example, when expression of PRKG1 protein or mRNA protein is greater (statistically significantly greater) in the presence of the candidate compound than in its
25 absence, the candidate compound is identified as a stimulator of PRKG1 protein or mRNA expression. Alternatively, when expression of PRKG1 protein or mRNA is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of PRKG1 protein or mRNA expression. The level of PRKG1 protein or mRNA expression in the cells can be determined by methods described below.
- 30 Such screening can be carried out either in a cell-free assay system or in an intact cell. Any cell which expresses PRKG1 polynucleotide can be used in a cell-based assay system. The PRKG1 polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Either a primary culture or an established cell line can be used.

Binding Assays

For binding assays, the test compound is preferably a small molecule which binds to and occupies the active site of PRKG1 polypeptide, thereby making the ligand binding site inaccessible to substrate such that normal biological activity is prevented. Examples of such small molecules

5 include, but are not limited to, small peptides or peptide-like molecules. Potential ligands which bind to a polypeptide of the invention include, but are not limited to, the natural ligands of known PRKG1 kinases and analogues or derivatives thereof.

In binding assays, either the test compound or the PRKG1 polypeptide can comprise a detectable label, such as a fluorescent, radioisotopic, chemiluminescent, or enzymatic label, such as

10 horseradish peroxidase, alkaline phosphatase, or luciferase. Detection of a test compound which is bound to PRKG1 polypeptide can then be accomplished, for example, by direct counting of radio-emission, by scintillation counting, or by determining conversion of an appropriate substrate to a detectable product. Alternatively, binding of a test compound to a PRKG1 polypeptide can be determined without labeling either of the interactants. For example, a microphysiometer can be
15 used to detect binding of a test compound with a PRKG1 polypeptide. A microphysiometer (*e.g.*, CytosensorTM) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a test compound and PRKG1 [Haseloff, (1988)].

20 Determining the ability of a test compound to bind to PRKG1 also can be accomplished using a technology such as real-time Bimolecular Interaction Analysis (BIA) [McConnell, (1992); Sjolander, (1991)]. BIA is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIACoreTM). Changes in the optical phenomenon surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological
25 molecules.

In yet another aspect of the invention, a PRKG1-like polypeptide can be used as a "bait protein" in a two-hybrid assay or three-hybrid assay [Szabo, (1995); U.S. 5,283,317], to identify other proteins which bind to or interact with PRKG1 and modulate its activity.

The two-hybrid system is based on the modular nature of most transcription factors, which consist
30 of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. For example, in one construct, polynucleotide encoding PRKG1 can be fused to a polynucleotide encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct a DNA sequence that encodes an unidentified protein ("prey" or "sample")

can be fused to a polynucleotide that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact *in vivo* to form an protein-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ), which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected, and cell colonies containing the functional transcription factor can be isolated and used to obtain the DNA sequence encoding the protein which interacts with PRKG1.

It may be desirable to immobilize either the PRKG1 (or polynucleotide) or the test compound to facilitate separation of the bound form from unbound forms of one or both of the interactants, as well as to accommodate automation of the assay. Thus, either the PRKG1-like polypeptide (or polynucleotide) or the test compound can be bound to a solid support. Suitable solid supports include, but are not limited to, glass or plastic slides, tissue culture plates, microtiter wells, tubes, silicon chips, or particles such as beads (including, but not limited to, latex, polystyrene, or glass beads). Any method known in the art can be used to attach PRKG1-like polypeptide (or polynucleotide) or test compound to a solid support, including use of covalent and non-covalent linkages, passive absorption, or pairs of binding moieties attached respectively to the polypeptide (or polynucleotide) or test compound and the solid support. Test compounds are preferably bound to the solid support in an array, so that the location of individual test compounds can be tracked. Binding of a test compound to PRKG1 (or a polynucleotide encoding for PRKG1) can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and microcentrifuge tubes.

In one embodiment, PRKG1 is a fusion protein comprising a domain that allows binding of PRKG1 to a solid support. For example, glutathione-S-transferase fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, Mo.) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and the non-adsorbed PRKG1; the mixture is then incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components. Binding of the interactants can be determined either directly or indirectly, as described above. Alternatively, the complexes can be dissociated from the solid support before binding is determined.

Other techniques for immobilizing proteins or polynucleotides on a solid support also can be used in the screening assays of the invention. For example, either PRKG1 (or a polynucleotide encoding PRKG1) or a test compound can be immobilized utilizing conjugation of biotin and

streptavidin. Biotinylated PRKG1 (or a polynucleotide encoding biotinylated PRKG1) or test compounds can be prepared from biotin-NHS (N-hydroxysuccinimide) using techniques well known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.) and immobilized in the wells of streptavidin-coated plates (Pierce Chemical). Alternatively, antibodies which specifically bind to PRKG1, polynucleotide, or a test compound, but which do not interfere with a desired binding site, such as the active site of PRKG1, can be derivatized to the wells of the plate. Unbound target or protein can be trapped in the wells by antibody conjugation.

Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies which specifically bind to PRKG1 polypeptide or test compound, enzyme-linked assays which rely on detecting an activity of PRKG1 polypeptide, and SDS gel electrophoresis under non-reducing conditions.

Screening for test compounds which bind to a PRKG1 polypeptide or polynucleotide also can be carried out in an intact cell. Any cell which comprises a PRKG1 polypeptide or polynucleotide can be used in a cell-based assay system. A PRKG1 polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Binding of the test compound to PRKG1 or a polynucleotide encoding PRKG1 is determined as described above.

Functional Assays

Test compounds can be tested for the ability to increase or decrease PRKG1 activity of a PRKG1 polypeptide. The PRKG1 activity can be measured, for example, using methods described in the specific examples, below. PRKG1 activity can be measured after contacting either a purified PRKG1 or an intact cell with a test compound. A test compound which decreases PRKG1 activity by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential agent for decreasing PRKG1 activity. A test compound which increases PRKG1 activity by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential agent for increasing PRKG1 activity.

Gene Expression

In another embodiment, test compounds which increase or decrease PRKG1 gene expression are identified. As used herein, the term "correlates with expression of a polynucleotide" indicates that the detection of the presence of nucleic acids, the same or related to a nucleic acid sequence encoding PRKG1, by northern analysis or realtime PCR is indicative of the presence of nucleic acids encoding PRKG1 in a sample, and thereby correlates with expression of the transcript from

the polynucleotide encoding PRKG1. The term "microarray", as used herein, refers to an array of distinct polynucleotides or oligonucleotides arrayed on a substrate, such as paper, nylon or any other type of membrane, filter, chip, glass slide, or any other suitable solid support. A PRKG1 polynucleotide is contacted with a test compound, and the expression of an RNA or polypeptide product of PRKG1 polynucleotide is determined. The level of expression of appropriate mRNA or polypeptide in the presence of the test compound is compared to the level of expression of mRNA or polypeptide in the absence of the test compound. The test compound can then be identified as a regulator of expression based on this comparison. For example, when expression of mRNA or polypeptide is greater in the presence of the test compound than in its absence, the test compound is identified as a stimulator or enhancer of the mRNA or polypeptide expression. Alternatively, when expression of the mRNA or polypeptide is less in the presence of the test compound than in its absence, the test compound is identified as an inhibitor of the mRNA or polypeptide expression.

The level of PRKG1 mRNA or polypeptide expression in the cells can be determined by methods well known in the art for detecting mRNA or polypeptide. Either qualitative or quantitative methods can be used. The presence of polypeptide products of PRKG1 polynucleotide can be determined, for example, using a variety of techniques known in the art, including immunochemical methods such as radioimmunoassay, Western blotting, and immunohistochemistry. Alternatively, polypeptide synthesis can be determined *in vivo*, in a cell culture, or in an *in vitro* translation system by detecting incorporation of labelled amino acids into PRKG1.

20 *Test Compounds*

Suitable test compounds for use in the screening assays of the invention can be obtained from any suitable source, e.g., conventional compound libraries. The test compounds can also be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds [Lam, (1997)]. Examples of methods for the synthesis of molecular libraries can be found in the art. Libraries of compounds may be presented in solution or on beads, bacteria, spores, plasmids or phage.

30 *Modeling of Regulators*

Computer modeling and searching technologies permit identification of compounds, or the improvement of already identified compounds, that can modulate PRKG1 expression or activity.

Having identified such a compound or composition, the active sites or regions are identified. Such sites might typically be the enzymatic active site, regulator binding sites, or ligand binding sites. The active site can be identified using methods known in the art including, for example, from the amino acid sequences of peptides, from the nucleotide sequences of nucleic acids, or from study of complexes of the relevant compound or composition with its natural ligand. In the latter case, chemical or X-ray crystallographic methods can be used to find the active site by finding where on the factor the complexed ligand is found.

Next, the three dimensional geometric structure of the active site is determined. This can be done by known methods, including X-ray crystallography, which can determine a complete molecular structure. On the other hand, solid or liquid phase NMR can be used to determine certain intramolecular distances. Any other experimental method of structure determination can be used to obtain partial or complete geometric structures. The geometric structures may be measured with a complexed ligand, natural or artificial, which may increase the accuracy of the active site structure determined.

- 15 If an incomplete or insufficiently accurate structure is determined, the methods of computer based numerical modeling can be used to complete the structure or improve its accuracy. Any recognized modeling method may be used, including parameterized models specific to particular biopolymers such as proteins or nucleic acids, molecular dynamics models based on computing molecular motions, statistical mechanics models based on thermal ensembles, or combined models.
- 20 For most types of models, standard molecular force fields, representing the forces between constituent atoms and groups, are necessary, and can be selected from force fields known in physical chemistry. The incomplete or less accurate experimental structures can serve as constraints on the complete and more accurate structures computed by these modeling methods.

Finally, having determined the structure of the active site, either experimentally, by modeling, or by a combination, candidate modulating compounds can be identified by searching databases containing compounds along with information on their molecular structure. Such a search seeks compounds having structures that match the determined active site structure and that interact with the groups defining the active site. Such a search can be manual, but is preferably computer assisted. These compounds found from this search are potential PRKG1 modulating compounds.

- 30 Alternatively, these methods can be used to identify improved modulating compounds from an already known modulating compound or ligand. The composition of the known compound can be modified and the structural effects of modification can be determined using the experimental and computer modeling methods described above applied to the new composition. The altered structure is then compared to the active site structure of the compound to determine if an improved

fit or interaction results. In this manner systematic variations in composition, such as by varying side groups, can be quickly evaluated to obtain modified modulating compounds or ligands of improved specificity or activity.

Therapeutic Indications and Methods

- 5 It was found by the present applicant that PRKG1 is expressed in various human tissues.

Neurology

CNS disorders include disorders of the central nervous system as well as disorders of the peripheral nervous system.

- 10 CNS disorders include, but are not limited to brain injuries, cerebrovascular diseases and their consequences, Parkinson's disease, corticobasal degeneration, motor neuron disease, dementia, including ALS, multiple sclerosis, traumatic brain injury, stroke, post-stroke, post-traumatic brain injury, and small-vessel cerebrovascular disease. Dementias, such as Alzheimer's disease, vascular dementia, dementia with Lewy bodies, frontotemporal dementia and Parkinsonism linked to chromosome 17, frontotemporal dementias, including Pick's disease, progressive nuclear palsy, 15 corticobasal degeneration, Huntington's disease, thalamic degeneration, Creutzfeld-Jakob dementia, HIV dementia, schizophrenia with dementia, and Korsakoff's psychosis, within the meaning of the definition are also considered to be CNS disorders.

- 20 Similarly, cognitive-related disorders, such as mild cognitive impairment, age-associated memory impairment, age-related cognitive decline, vascular cognitive impairment, attention deficit disorders, attention deficit hyperactivity disorders, and memory disturbances in children with learning disabilities are also considered to be CNS disorders.

- 25 Pain, within the meaning of this definition, is also considered to be a CNS disorder. Pain can be associated with CNS disorders, such as multiple sclerosis, spinal cord injury, sciatica, failed back surgery syndrome, traumatic brain injury, epilepsy, Parkinson's disease, post-stroke, and vascular lesions in the brain and spinal cord (e.g., infarct, hemorrhage, vascular malformation). Non-central neuropathic pain includes that associated with post mastectomy pain, phantom feeling, reflex sympathetic dystrophy (RSD), trigeminal neuralgia/radioculopathy, post-surgical pain, HIV/AIDS related pain, cancer pain, metabolic neuropathies (e.g., diabetic neuropathy, vasculitic neuropathy secondary to connective tissue disease), paraneoplastic polyneuropathy associated, for example, 30 with carcinoma of lung, or leukemia, or lymphoma, or carcinoma of prostate, colon or stomach, trigeminal neuralgia, cranial neuralgias, and post-herpetic neuralgia. Pain associated with peripheral nerve damage, central pain (i.e. due to cerebral ischemia) and various chronic pain i.e.,

lumbago, back pain (low back pain), inflammatory and/or rheumatic pain. Headache pain (for example, migraine with aura, migraine without aura, and other migraine disorders), episodic and chronic tension-type headache, tension-type like headache, cluster headache, and chronic paroxysmal hemicrania are also CNS disorders.

- 5 Visceral pain such as pancreatitis, intestinal cystitis, dysmenorrhea, irritable Bowel syndrome, Crohn's disease, biliary colic, ureteral colic, myocardial infarction and pain syndromes of the pelvic cavity, e.g., vulvodynia, orchialgia, urethral syndrome and protatodynia are also CNS disorders.

10 Also considered to be a disorder of the nervous system are acute pain, for example postoperative pain, and pain after trauma.

The human PRKG1 is highly expressed in the following brain tissues: fetal brain, brain, Alzheimer brain, cerebellum, Alzheimer brain cerebellum, cerebellum (left), cerebral cortex, Alzheimer cerebral cortex, frontal lobe, occipital lobe, parietal lobe, temporal lobe, postcentral gyrus, cerebral peduncles, nucleus accumbens, putamen, hippocampus, thalamus, posteroverentral thalamus, 15 dorsalmedial thalamus, spinal cord, spinal cord (ventral horn), spinal cord (dorsal horn), neuroblastoma SH-SY5Y cells, astrocytes, retina. The expression in brain tissues and in particular the differential expression between diseased tissue Alzheimer brain and healthy tissue brain, between diseased tissue Alzheimer brain cerebellum and healthy tissue , between diseased tissue Alzheimer cerebral cortex and healthy tissue cerebral cortex demonstrates that the human PRKG1 20 or mRNA can be utilized to diagnose nervous system diseases. Additionally the activity of the human PRKG1 can be modulated to treat nervous system diseases.

Cardiovascular Disorders

Heart failure is defined as a pathophysiological state in which an abnormality of cardiac function is responsible for the failure of the heart to pump blood at a rate commensurate with the requirement 25 of the metabolizing tissue. It includes all forms of pumping failures such as high-output and low-output, acute and chronic, right-sided or left-sided, systolic or diastolic, independent of the underlying cause.

Myocardial infarction (MI) is generally caused by an abrupt decrease in coronary blood flow that follows a thrombotic occlusion of a coronary artery previously narrowed by arteriosclerosis. MI 30 prophylaxis (primary and secondary prevention) is included as well as the acute treatment of MI and the prevention of complications.

Ischemic diseases are conditions in which the coronary flow is restricted resulting in a perfusion which is inadequate to meet the myocardial requirement for oxygen. This group of diseases includes stable angina, unstable angina and asymptomatic ischemia.

Arrhythmias include all forms of atrial and ventricular tachyarrhythmias, atrial tachycardia, atrial flutter, atrial fibrillation, atrio-ventricular reentrant tachycardia, preexcitation syndrome, ventricular tachycardia, ventricular flutter, ventricular fibrillation, as well as bradycardic forms of arrhythmias.

Hypertensive vascular diseases include primary as well as all kinds of secondary arterial hypertension, renal, endocrine, neurogenic, others. The genes may be used as drug targets for the treatment of hypertension as well as for the prevention of all complications arising from cardiovascular diseases.

Peripheral vascular diseases are defined as vascular diseases in which arterial and/or venous flow is reduced resulting in an imbalance between blood supply and tissue oxygen demand. It includes chronic peripheral arterial occlusive disease (PAOD), acute arterial thrombosis and embolism, inflammatory vascular disorders, Raynaud's phenomenon and venous disorders.

Atherosclerosis is a cardiovascular disease in which the vessel wall is remodeled, compromising the lumen of the vessel. The atherosclerotic remodeling process involves accumulation of cells, both smooth muscle cells and monocyte/macrophage inflammatory cells, in the intima of the vessel wall. These cells take up lipid, likely from the circulation, to form a mature atherosclerotic lesion.

Although the formation of these lesions is a chronic process, occurring over decades of an adult human life, the majority of the morbidity associated with atherosclerosis occurs when a lesion ruptures, releasing thrombogenic debris that rapidly occludes the artery. When such an acute event occurs in the coronary artery, myocardial infarction can ensue, and in the worst case, can result in death.

The formation of the atherosclerotic lesion can be considered to occur in five overlapping stages such as migration, lipid accumulation, recruitment of inflammatory cells, proliferation of vascular smooth muscle cells, and extracellular matrix deposition. Each of these processes can be shown to occur in man and in animal models of atherosclerosis, but the relative contribution of each to the pathology and clinical significance of the lesion is unclear.

Thus, a need exists for therapeutic methods and agents to treat cardiovascular pathologies, such as atherosclerosis and other conditions related to coronary artery disease.

Cardiovascular diseases include but are not limited to disorders of the heart and the vascular system like congestive heart failure, myocardial infarction, ischemic diseases of the heart, all kinds of atrial and ventricular arrhythmias, hypertensive vascular diseases, peripheral vascular diseases, and atherosclerosis.

- 5 Too high or too low levels of fats in the bloodstream, especially cholesterol, can cause long-term problems. The risk to develop atherosclerosis and coronary artery or carotid artery disease (and thus the risk of having a heart attack or stroke) increases with the total cholesterol level increasing. Nevertheless, extremely low cholesterol levels may not be healthy. Examples of disorders of lipid metabolism are hyperlipidemia (abnormally high levels of fats (cholesterol, triglycerides, or both) 10 in the blood, may be caused by family history of hyperlipidemia), obesity, a high-fat diet, lack of exercise, moderate to high alcohol consumption, cigarette smoking, poorly controlled diabetes, and an underactive thyroid gland), hereditary hyperlipidemias (type I hyperlipoproteinemia (familial hyperchylomicronemia), type II hyperlipoproteinemia (familial hypercholesterolemia), type III hyperlipoproteinemia, type IV hyperlipoproteinemia, or type V hyperlipoproteinemia), hypo- 15 lipoproteinemia, lipidoses (caused by abnormalities in the enzymes that metabolize fats), Gaucher's disease, Niemann-Pick disease, Fabry's disease, Wolman's disease, cerebrotendinous xanthomatosis, sitosterolemia, Refsum's disease, or Tay-Sachs disease.

- Kidney disorders may lead to hypertension or hypotension. Examples for kidney problems possibly leading to hypertension are renal artery stenosis, pyelonephritis, glomerulonephritis, kidney 20 tumors, polycistic kidney disease, injury to the kidney, or radiation therapy affecting the kidney. Excessive urination may lead to hypotension.

The human PRKG1 is highly expressed in the following cardiovascular related tissues: heart, pericardium, heart atrium (right), heart atrium (left), heart ventricle (left), heart ventricle (right), heart apex, Purkinje fibers, interventricular septum, aorta, aorta sclerotic, artery, coronary artery, 25 coronary artery sclerotic, pulmonary artery, carotid artery, mesenteric artery, vein, pulmonic valve, coronary artery endothel cells, coronary artery smooth muscle primary cells, aortic smooth muscle cells, pulmonary artery smooth muscle cells, aortic endothel cells, adrenal gland, liver, liver liver cirrhosis, adipose. Expression in the above mentioned tissues and in particular the differential expression between diseased tissue aorta sclerotic and healthy tissue , between diseased tissue 30 coronary artery sclerotic and healthy tissue coronary artery demonstrates that the human PRKG1 or mRNA can be utilized to diagnose of cardiovascular diseases. Additionally the activity of the human PRKG1 can be modulated to treat cardiovascular diseases.

The human PRKG1 is highly expressed in liver tissues: liver, liver liver cirrhosis. Expression in liver tissues demonstrates that the human PRKG1 or mRNA can be utilized to diagnose of

dyslipidemia disorders as an cardiovascular disorder. Additionally the activity of the human PRKG1 can be modulated to treat - but not limited to - dyslipidemia disorders.

The human PRKG1 is highly expressed in adipose tissues. Expression in adipose demonstrates that the human PRKG1 or mRNA can be utilized to diagnose of dyslipidemia diseases as an 5 cardiovascular disorder. Additionally the activity of the human PRKG1 can be modulated to treat - but not limited to - dyslipidemia diseases.

The human PRKG1 is highly expressed in adrenal gland. Expression in adrenal gland tissues demonstrates that the human PRKG1 or mRNA can be utilized to diagnose of blood pressure disorders as an cardiovascular disorder. Additionally the activity of the human PRKG1 can be 10 modulated to treat - but not limited to - blood pressure disorders as hypertension or hypotension.

Hematological Disorders

Hematological disorders comprise diseases of the blood and all its constituents as well as diseases of organs and tissues involved in the generation or degradation of all the constituents of the blood. They include but are not limited to 1) Anemias, 2) Myeloproliferative Disorders, 3) Hemorrhagic 15 Disorders, 4) Leukopenia, 5) Eosinophilic Disorders, 6) Leukemias, 7) Lymphomas, 8) Plasma Cell Dyscrasias, 9) Disorders of the Spleen in the course of hematological disorders. Disorders according to 1) include, but are not limited to anemias due to defective or deficient hem synthesis, deficient erythropoiesis. Disorders according to 2) include, but are not limited to polycythemia vera, tumor-associated erythrocytosis, myelofibrosis, thrombocythemia. Disorders according to 3)

20 include, but are not limited to vasculitis, thrombocytopenia, heparin-induced thrombocytopenia, thrombotic thrombocytopenic purpura, hemolytic-uremic syndrome, hereditary and acquired disorders of platelet function, hereditary coagulation disorders. Disorders according to 4) include, but are not limited to neutropenia, lymphocytopenia. Disorders according to 5) include, but are not limited to hypereosinophilia, idiopathic hypereosinophilic syndrome. Disorders according to 6)

25 include, but are not limited to acute myeloic leukemia, acute lymphoblastic leukemia, chronic myelocytic leukemia, chronic lymphocytic leukemia, myelodysplastic syndrome. Disorders according to 7) include, but are not limited to Hodgkin's disease, non-Hodgkin's lymphoma, Burkitt's lymphoma, mycosis fungoides cutaneous T-cell lymphoma. Disorders according to 8)

30 include, but are not limited to multiple myeloma, macroglobulinemia, heavy chain diseases. In extension of the preceding idiopathic thrombocytopenic purpura, iron deficiency anemia, megaloblastic anemia (vitamin B12 deficiency), aplastic anemia, thalassemia, malignant lymphoma bone marrow invasion, malignant lymphoma skin invasion, hemolytic uremic syndrome, giant platelet disease are considered to be hematological diseases too.

The human PRKG1 is highly expressed in the following tissues of the hematological system: leukocytes (peripheral blood), bone marrow, erythrocytes, bone marrow CD34+ cells, cord blood CD34+ cells, spleen, spleen liver cirrhosis. The expression in the above mentioned tissues and in particular the differential expression between diseased tissue spleen liver cirrhosis and healthy 5 tissue spleen demonstrates that the human PRKG1 or mRNA can be utilized to diagnose of hematological diseases. Additionally the activity of the human PRKG1 can be modulated to treat hematological disorders.

Gastrointestinal and Liver Diseases

Gastrointestinal diseases comprise primary or secondary, acute or chronic diseases of the organs of 10 the gastrointestinal tract which may be acquired or inherited, benign or malignant or metaplastic, and which may affect the organs of the gastrointestinal tract or the body as a whole. They comprise but are not limited to 1) disorders of the esophagus like achalasia, vigorous achalasia, dysphagia, cricopharyngeal incoordination, pre-esophageal dysphagia, diffuse esophageal spasm, globus sensation, Barrett's metaplasia, gastroesophageal reflux, 2) disorders of the stomach and 15 duodenum like functional dyspepsia, inflammation of the gastric mucosa, gastritis, stress gastritis, chronic erosive gastritis, atrophy of gastric glands, metaplasia of gastric tissues, gastric ulcers, duodenal ulcers, neoplasms of the stomach, 3) disorders of the pancreas like acute or chronic pancreatitis, insufficiency of the exocrine or endocrine tissues of the pancreas like steatorrhea, diabetes, neoplasms of the exocrine or endocrine pancreas like 3.1) multiple endocrine neoplasia 20 syndrome, ductal adenocarcinoma, cystadenocarcinoma, islet cell tumors, insulinoma, gastrinoma, carcinoid tumors, glucagonoma, Zollinger-Ellison syndrome, Vipoma syndrome, malabsorption syndrome, 4) disorders of the bowel like chronic inflammatory diseases of the bowel, Crohn's disease, ileus, diarrhea and constipation, colonic inertia, megacolon, malabsorption syndrome, ulcerative colitis, 4.1) functional bowel disorders like irritable bowel syndrome, 4.2) neoplasms of 25 the bowel like familial polyposis, adenocarcinoma, primary malignant lymphoma, carcinoid tumors, Kaposi's sarcoma, polyps, cancer of the colon and rectum.

Liver diseases comprise primary or secondary, acute or chronic diseases or injury of the liver which may be acquired or inherited, benign or malignant, and which may affect the liver or the body as a whole. They comprise but are not limited to disorders of the bilirubin metabolism, 30 jaundice, syndromes of Gilbert's, Crigler-Najjar, Dubin-Johnson and Rotor; intrahepatic cholestasis, hepatomegaly, portal hypertension, ascites, Budd-Chiari syndrome, portal-systemic encephalopathy, fatty liver, steatosis, Reye's syndrome, liver diseases due to alcohol, alcoholic hepatitis or cirrhosis, fibrosis and cirrhosis, fibrosis and cirrhosis of the liver due to inborn errors of metabolism or exogenous substances, storage diseases, syndromes of Gaucher's, Zellweger's,

Wilson's - disease, acute or chronic hepatitis, viral hepatitis and its variants, inflammatory conditions of the liver due to viruses, bacteria, fungi, protozoa, helminths; drug induced disorders of the liver, chronic liver diseases like primary sclerosing cholangitis, alpha₁-antitrypsin-deficiency, primary biliary cirrhosis, postoperative liver disorders like postoperative intrahepatic cholestasis, hepatic granulomas, vascular liver disorders associated with systemic disease, benign or malignant neoplasms of the liver, disturbance of liver metabolism in the new-born or prematurely born.

The human PRKG1 is highly expressed in the following tissues of the gastroenterological system: esophagus, esophagus tumor, stomach, stomach tumor, colon, colon tumor, ileum, ileum tumor, rectum, salivary gland, liver, liver liver cirrhosis. The expression in the above mentioned tissues and in particular the differential expression between diseased tissue liver liver cirrhosis and healthy tissue liver demonstrates that the human PRKG1 or mRNA can be utilized to diagnose of gastroenterological disorders. Additionally the activity of the human PRKG1 can be modulated to treat gastroenterological disorders.

15 *Endocrine System and Hormones*

The endocrine system consists of a group of organs whose main function is to produce and secrete hormones directly into the bloodstream. The major organs of the endocrine system are the hypothalamus, the pituitary gland, thyroid gland, the parathyroid glands, the islets of the pancreas, the adrenal glands, the testes, and the ovaries.

20 The hypothalamus secretes several hormones that stimulate the pituitary: Some trigger the release of pituitary hormones; others suppress the release of pituitary hormones.

The pituitary gland coordinates many functions of the other endocrine glands, but some pituitary hormones have direct effects.

25 The insulin-secreting cells of the pancreas respond to glucose and fatty acids. Parathyroid cells respond to calcium and phosphate. The adrenal medulla (part of the adrenal gland) responds to direct stimulation by the parasympathetic nervous system.

When endocrine glands malfunction, hormone levels in the blood can become abnormally high or low, disrupting body functions. Many disorders are caused by malfunction of the endocrine system or hormones. Examples of such disorders are presented in the following.

30 Diabetes mellitus is a disorder in which blood levels of glucose are abnormally high because the body doesn't release or use insulin adequately.

People with type I diabetes mellitus (insulin-dependent diabetes) produce little or no insulin at all. In type I diabetes more than 90 percent of the insulin-producing cells (beta cells) of the pancreas are permanently destroyed. The resulting insulin deficiency is severe, and to survive, a person with type I diabetes must regularly inject insulin.

- 5 In type II diabetes mellitus (non-insulin-dependent diabetes) the body develops resistance to insulin effects, resulting in a relative insulin deficiency.

The pancreas has two major functions: to secrete fluid containing digestive enzymes into the duodenum and to secrete the hormones insulin and glucagon. Chronic pancreatitis is a long-standing inflammation of the pancreas. Eventually, the insulin-secreting cells of the pancreas may

- 10 be destroyed, gradually leading to diabetes. An insulinoma is a rare type of pancreatic tumor that secretes insulin. The symptoms of an insulinoma result from low blood glucose levels. A gastrinoma is a pancreatic tumor that produces excessive levels of the hormone gastrin, which stimulates the stomach to secrete acid and enzymes, causing peptic ulcers. The excess gastrin secreted by the gastrinoma causes symptoms, called the Zollinger-Ellison syndrome. A
15 glucagonoma is a tumor that produces the hormone glucagon, which raises the level of glucose in the blood and produces a distinctive rash.

Diabetes insipidus is a disorder in which insufficient levels of antidiuretic hormone cause excessive thirst (polydipsia) and excessive production of very dilute urine (polyuria). Diabetes insipidus results from the decreased production of antidiuretic hormone (vasopressin).

- 20 The body has two adrenal glands. The medulla of the adrenal glands secretes hormones such as adrenaline (epinephrine) that affect blood pressure, heart rate, sweating, and other activities also regulated by the sympathetic nervous system. The cortex secretes many different hormones, including corticosteroids (cortisone-like hormones), androgens (male hormones), and mineralocorticoids, which control blood pressure and the levels of salt and potassium in the body.
25 A disease characterized by underactive adrenal glands is Addison's disease (adrenocortical insufficiency).

Several disorders are characterized by overactive Adrenal Glands. The causes can be changes in the adrenal glands themselves or overstimulation by the pituitary gland. Examples of these diseases are listed in the following.

- 30 Overproduction of androgenic steroids (testosterone and similar hormones, leads to virilization), overproduction of corticosteroids (causes could be tumors of the pituitary or the adrenal gland, results in Cushing's syndrome), Nelson's syndrome (developed by people who have both adrenal

glands removed, characterized by an enlargement of the pituitary gland), Overproduction of aldosterone (hyperaldosteronism), Conn's syndrome (hyperaldosteronism caused by a tumor), pheochromocytoma (a tumor that originating from the adrenal gland's chromaffin cells, causing overproduction of catecholamines),

- 5 The thyroid is a small gland located under the Adam's apple. It secretes thyroid hormones, which control the metabolic rate. The thyroid gland traps iodine and processes it into thyroid hormones. The euthyroid sick syndrome is characterized by lack of conversion of the T4 form of thyroid hormone to the T3 form. Hyperthyroidism (overactive thyroid gland, production of too much hormone) may have several causes. Thyroiditis (an inflammation of the thyroid gland), typically
10 leads to a phase of hyperthyroidism. The inflammation may damage the thyroid gland, so that in later stages the disease is characterized by transient or permanent underactivity (hypothyroidism). Toxic thyroid nodules (adenomas) often produce thyroid hormone in large quantities. Toxic multinodular goiter (Plummer's disease) is a disorder in which there are many nodules. Graves' disease (toxic diffuse goiter) is believed to be caused by an antibody that stimulates the thyroid to
15 produce too much thyroid hormone. In toxic nodular goiter, one or more nodules in the thyroid produce too much thyroid hormone and aren't under the control of thyroid-stimulating hormone. Secondary hyperthyroidism may (rarely) be caused by a pituitary tumor that secretes too much thyroid-stimulating hormone, by resistance of the pituitary to thyroid hormone, which results in the pituitary gland secreting too much thyroid-stimulating hormone, or by a hydatidiform mole in
20 women. Thyroid storm is a sudden extreme overactivity of the thyroid gland is a life-threatening emergency requiring prompt treatment.

Hypothyroidism is a condition in which the thyroid gland is underactive and produces too little thyroid hormone. Very severe hypothyroidism is called myxedema. In Hashimoto's thyroiditis (autoimmune thyroiditis) the thyroid gland is often enlarged, and hypothyroidism results because
25 the gland's functioning areas are gradually destroyed. Rarer causes of hypothyroidism include some inherited disorders which are caused by abnormalities of the enzymes in thyroid cells. In other rare disorders, either the hypothalamus or the pituitary gland fails to secrete enough of the hormone needed to stimulate normal thyroid function.

Other examples of Thyroiditis are silent lymphocytic thyroiditis, Hashimoto's thyroiditis, or
30 subacute granulomatous thyroiditis.

Thyroid cancer is any one of four main types of malignancy of the thyroid: papillary, follicular, anaplastic, or medullary.

The pituitary is a pea-sized gland that sits in a bony structure (sella turcica) at the base of the brain. The sella turcica protects the pituitary but allows very little room for expansion. If the pituitary enlarges, it tends to push upward, often pressing on the areas of the brain that carry signals from the eyes, possibly resulting in headaches or impaired vision. The pituitary gland has two distinct parts: the anterior (front) and the posterior (back) lobes. The anterior lobe produces (secretes) hormones that ultimately control the function of the thyroid gland, adrenal glands, and reproductive organs (ovaries and testes); milk production (lactation) in the breasts; and overall body growth. It also produces hormones that cause the skin to darken and that inhibit pain sensations. The posterior lobe produces hormones that regulate water balance, stimulate the let-down of milk from the breasts in lactating women, and stimulate contractions of the uterus.

Examples for disorders of the pituitary gland are Empty Sella Syndrome; hypopituitarism (an underactive pituitary gland); acromegaly, which is excessive growth caused by oversecretion of growth hormone, which is almost always caused by a benign pituitary tumor (adenoma); galactorrhea, which is the production of breast milk in men or in women who aren't breastfeeding, in both sexes, the most common cause of galactorrhea is a prolactin-producing tumor (prolactinoma) in the pituitary gland.

The human PRKG1 is highly expressed in the following tissues of the endocrinological system: adrenal gland, thyroid, pancreas, pancreas liver cirrhosis. The expression in the above mentioned tissues and in particular the differential expression between diseased tissue pancreas liver cirrhosis and healthy tissue pancreas demonstrates that the human PRKG1 or mRNA can be utilized to diagnose of endocrinological disorders. Additionally the activity of the human PRKG1 can be modulated to treat endocrinological disorders.

Dermatologic Disorders

The skin serves several functions. It's a multi-layered organ system that builds an effective protective cover and regulates body temperature, senses painful and pleasant stimuli, keeps substances from entering the body, and provides a shield from the sun's harmful effects. Skin color, texture, and folds help mark people as individuals. Thus, skin disorders or diseases often have important consequences for physical and mental health. Skin disorders include, but are not limited to the conditions described in the following.

30 Itching (pruritus) is a sensation that instinctively demands scratching, which may be caused by a skin condition or a systemic disease.

Superficial Skin Disorders affect the uppermost layer of the skin, the stratum corneum or the keratin layer, and it consists of many layers of flattened, dead cells and acts as a barrier to protect the underlying tissue from injury and infection. Disorders of the superficial skin layers involve the stratum corneum and deeper layers of the epidermis.

- 5 Examples of superficial skin disorders are provided in the following.

Dry skin often occurs in people past middle age, severe dry skin (ichthyosis) results from an inherited scaling disease, such as ichthyosis vulgaris or epidermolytic hyperkeratosis. Ichthyosis also results from nonhereditary disorders, such as leprosy, underactive thyroid, lymphoma, AIDS, and sarcoidosis.

- 10 Keratosis pilaris is a common disorder in which dead cells shed from the upper layer of skin and form plugs that fill the openings of hair follicles.

A callus is an area on the stratum corneum or keratin layer, that becomes abnormally thick in response to repeated rubbing.

A corn is a pea-sized, thickened area of keratin that occurs on the feet.

- 15 Psoriasis is a chronic, recurring disease recognizable by silvery scaling bumps and various-sized plaques (raised patches). An abnormally high rate of growth and turnover of skin cells causes the scaling.

Pityriasis rosea is a mild disease that causes scaly, rose-colored, inflamed skin. Pityriasis rosea is possibly caused by an infectious agent, although none has been identified.

- 20 Lichen planus, a recurring itchy disease, starts as a rash of small discrete bumps that then combine and become rough, scaly plaques (raised patches).

Dermatitis (eczema) is an inflammation of the upper layers of the skin, causing blisters, redness, swelling, oozing, scabbing, scaling, and usually itching.

Forms of dermatitis are contact dermatitis, or chronic dermatitis of the hands and feet, e.g.

- 25 Pompholyx.

Further examples of dermatitic disorders are atopic dermatitis, seborrheic dermatitis, nummular dermatitis, generalized exfoliative dermatitis, stasis dermatitis, or localized scratch dermatitis (lichen simplex chronicus, neurodermatitis).

Other skin disorders are caused by inflammation. The skin can break out in a variety of rashes, sores, and blisters. Some skin eruptions can even be life threatening.

Drug rashes are side effects of medications, mainly allergic reactions to medications.

Toxic epidermal necrolysis is a life-threatening skin disease in which the top layer of the skin peels off in sheets. This condition can be caused by a reaction to a drug, or by some other serious disease.

Erythema multiforme, often caused by herpes simplex is a disorder characterized by patches of red, raised skin that often look like targets and usually are distributed symmetrically over the body.

Erythema nodosum is an inflammatory disorder that produces tender red bumps (nodules) under the skin, most often over the shins but occasionally on the arms and other areas.

Granuloma annulare is a chronic skin condition of unknown cause in which small, firm, raised bumps form a ring with normal or slightly sunken skin in the center.

Some skin disorders are characterized as blistering diseases. Three autoimmune diseases-- pemphigus, bullous pemphigoid, and dermatitis herpetiformis--are among the most serious.

Pemphigus is an uncommon, sometimes fatal, disease in which blisters (bullae) of varying sizes break out on the skin, the lining of the mouth, and other mucous membranes.

Bullous pemphigoid is an autoimmune disease that causes blistering.

Dermatitis herpetiformis is an autoimmune disease in which clusters of intensely itchy, small blisters and hive-like swellings break out and persist. In people with the disease, proteins in wheat, rye, barley, and oat products activate the immune system, which attacks parts of the skin and somehow causes the rash and itching.

Sweating disorders also belong to skin disorders.

Prickly heat is an itchy skin rash caused by trapped sweat.

Excessive sweating (hyperhidrosis) may affect the entire surface of the skin, but often it's limited to the palms, soles, armpits, or groin. The affected area is often pink or bluish white, and in severe cases the skin may be cracked, scaly, and soft, especially on the feet.

Skin disorders can affect the sebaceous glands. The sebaceous glands, which secrete oil onto the skin, lie in the dermis, the skin layer just below the surface layer (epidermis). Sebaceous gland disorders include acne, rosacea, perioral dermatitis, and sebaceous cysts.

- 5 Acne is a common skin condition in which the skin pores become clogged, leading to pimples and inflamed, infected abscesses (collections of pus). Acne tends to develop in teenagers.

Acne is further subdivided in superficial acne or deep acne.

Rosacea is a persistent skin disorder that produces redness, tiny pimples, and broken blood vessels, usually on the central area of the face.

Perioral dermatitis is a red, often bumpy rash around the mouth and on the chin.

- 10 A sebaceous cyst (keratinous cyst) is a slow-growing bump containing dead skin, skin excretions, and other skin particles. These cysts may be small and can appear anywhere.

Hair Disorders also are skin disorders. Hair disorders include excessive hairiness, baldness, and ingrown beard hairs.

- 15 The skin can be infected by bacteria. Bacterial skin infections can range in seriousness from minor acne to a life-threatening condition, such as staphylococcal scalded skin syndrome. The most common bacterial skin infections are caused by *Staphylococcus* and *Streptococcus*. Risk factors for skin infections are for example diabetes, AIDS or skin lesions.

Impetigo is a skin infection, caused by *Staphylococcus* or *Streptococcus*, leading to the formation of small pus-filled blisters (pustules).

- 20 Folliculitis is an inflammation of the hair follicles caused by infection with *Staphylococcus*. The infection damages the hairs, which can be easily pulled out.

Boils (furuncles) are large, tender, swollen, raised areas caused by staphylococcal infection around hair follicles.

- 25 Carbuncles are clusters of boils that result in extensive sloughing of skin and scar formation. Carbuncles develop and heal more slowly than single boils and may lead to fever and fatigue.

Erysipelas is a skin infection caused by *Streptococcus*. A shiny, red, slightly swollen, tender rash develops, often with small blisters. Lymph nodes around the infected area may become enlarged and painful.

Cellulitis is a spreading infection in, and sometimes beneath, the deep layers of the skin. Cellulitis most often results from a streptococcal infection or a staphylococcal infection. However, many other bacteria can also cause cellulitis.

- 5 Paronychia is an infection around the edge of a fingernail or toenail. Paronychia can be caused by many different bacteria, including Pseudomonas and Proteus, and by fungi, such as Candida.

Staphylococcal scalded skin syndrome is a widespread skin infection that can lead to toxic shock syndrome, in which the skin peels off as though burned. Certain types of staphylococci produce a toxic substance that causes the top layer of skin (epidermis) to split from the rest of the skin.

- 10 Erythrasma is an infection of the top layers of the skin by the bacterium *Corynebacterium minutissimum*.

Skin infections are often caused by fungi. Fungi that infect the skin (dermatophytes) live only in the dead, topmost layer (stratum corneum) and don't penetrate deeper. Some fungal infections cause no symptoms or produce only a small amount of irritation, scaling, and redness. Other fungal infections cause itching, swelling, blisters, and severe scaling.

- 15 Ringworm is a fungal skin infection caused by several different fungi and generally classified by its location on the body.

Examples are Athlete's foot (foot ringworm, caused by either *Trichophyton* or *Epidermophyton*), jock itch (groin ringworm, can be caused by a variety of fungi and yeasts), scalp ringworm, caused by *Trichophyton* or *Microsporum*, nail ringworm and body ringworm (caused by *Trichophyton*).

- 20 Candidiasis (yeast infection, moniliasis) is an infection by the yeast *Candida*. *Candida* usually infects the skin and mucous membranes, such as the lining of the mouth and vagina. Rarely, it invades deeper tissues as well as the blood, causing life-threatening systemic candidiasis. The following types of candida infections can be distinguished: Infections in skinfolds (intertriginous infections), vaginal and penile candida infections (vulvovaginitis), thrush, Perlèche (candida infection at the corners of the mouth), candidal paronychia (candida growing in the nail beds, produces painful swelling and pus).

Tinea versicolor is a fungal infection that causes white to light brown patches on the skin.

The skin can also be affected by parasites, mainly tiny insects or worms.

- Scabies is a mite infestation that produces tiny reddish pimples and severe itching. Scabies is 30 caused by the itch mite *Sarcoptes scabiei*.

Lice infestation (pediculosis) causes intense itching and can affect almost any area of the skin. Head lice and pubic lice are two different species.

Creeping eruption (cutaneous larva migrans) is a hookworm infection transmitted from warm, moist soil to exposed skin. The infection is caused by a hookworm that normally inhabits dogs and

5 cats.

Many types of viruses invade the skin. The medically important ones cause warts and cold sores (fever blisters) on the lip. Warts are caused by the papillomavirus, and cold sores are caused by the herpes simplex virus. Another important group of viruses that infect the skin belongs to the poxvirus family. Chickenpox remains a common childhood infection. A poxvirus also causes

10 molluscum contagiosum, which is an infection of the skin by a poxvirus that causes skin-colored, smooth, waxy bumps.

Sunlight can cause severe skin damage. Sunburn results from an overexposure to ultraviolet B (UVB) rays. Some sunburned people develop a fever, chills, and weakness, and those with very bad sunburns even may go into shock--low blood pressure, and fainting.

15 People who are in the sun a lot have an increased risk of skin cancers, including squamous cell carcinoma, basal cell carcinoma, and to some degree, malignant melanoma.

Drugs, among other causes, can cause skin photosensitivity reactions which can occur after only a few minutes of sun exposure. These reactions include redness, peeling, hives, blisters, and thickened, scaling patches (photosensitivity).

20 Some skin disorders are characterized as Pigment Disorders.

Albinism is a rare, inherited disorder in which no melanin is formed.

Vitiligo is a condition in which a loss of melanocytes results in smooth, whitish patches of skin, which may occur after unusual physical trauma and tends to occur with certain other diseases, including Addison's disease, diabetes, pernicious anemia, and thyroid disease.

25 Tinea versicolor is a fungal infection of the skin that sometimes results in hyperpigmentation.

Melasma appears on the face (usually the forehead, cheeks, temples, and jaws) as a roughly symmetric group of dark brown patches of pigmentation that are often clearly delineated.

Skin growths, which are abnormal accumulations of different types of cells, may be present at birth or develop later. Noncancerous (benign) growth and cancerous (malignant) growth types are distinguished.

- 5 Moles (nevi) are small, usually dark, skin growths that develop from pigment-producing cells in the skin (melanocytes). Most moles are harmless. However, noncancerous moles can develop into malignant melanoma.

Skin tags are soft, small, flesh-colored or slightly darker skin flaps that appear mostly on the neck, in the armpits, or in the groin.

Lipomas are soft deposits of fatty material that grow under the skin, causing round or oval lumps.

- 10 Angiomas are collections of abnormally dense blood or lymph vessels that are usually located in and below the skin and that cause red or purple discolorations.

Examples of angiomas are port-wine stains, strawberry marks, cavernous hemangiomas, spider angiomas, and lymphangiomas.

- 15 Pyogenic granulomas are scarlet, brown, or blue-black slightly raised areas caused by increased growth of capillaries (the smallest blood vessels) and swelling of the surrounding tissue.

Seborrheic keratoses (sometimes called seborrheic warts) are flesh-colored, brown, or black growths that can appear anywhere on the skin.

Dermatofibromas are small, red-to-brown bumps (nodules) that result from an accumulation of fibroblasts, the cells that populate the soft tissue under the skin.

- 20 Keratoacanthomas are round, firm, usually flesh-colored growths that have an unusual central crater containing a pasty material.

Keloids are smooth, shiny, slightly pink, often dome-shaped, proliferative growths of fibrous tissue that form over areas of injury or over surgical wounds.

Skin cancer is the most common form of cancer, but most types of skin cancers are curable.

- 25 Basal cell carcinoma is a cancer that originates in the lowest layer of the epidermis.

Squamous cell carcinoma is cancer that originates in the middle layer of the epidermis.

Bowen's disease is a form of squamous cell carcinoma that's confined to the epidermis and hasn't yet invaded the underlying dermis.

Melanoma is a cancer that originates in the pigment-producing cells of the skin (melanocytes).

Kaposi's sarcoma is a cancer that originates in the blood vessels, usually of the skin.

Paget's disease is a rare type of skin cancer that looks like an inflamed, reddened patch of skin (dermatitis); it originates in glands in or under the skin.

- 5 The human PRKG1 is highly expressed in the following dermatological tissues: skin. The expression in the above mentioned tissues demonstrates that the human PRKG1 or mRNA can be utilized to diagnose of dermatological diseases. Additionally the activity of the human PRKG1 can be modulated to treat those diseases.

Musculoskeletal Diseases

- 10 Components of the musculoskeletal system are skeleton, muscles, tendons, ligaments, and other components of joints. Disorders of the musculoskeletal system often cause chronic pain and physical disability. They range from injuries, infections, inflammation or other types of disorders. Examples of musculoskeletal disorders are presented in the following.

- 15 Examples are osteoporosis, postmenopausal osteoporosis, senile osteoporosis, secondary osteoporosis, idiopathic juvenile osteoporosis, Paget's disease of the bone, osteochondromas (osteocartilaginous exostoses), tumors of the bone (benign chondromas, chondroblastomas, chondromyxoid fibromas, osteoid osteomas, giant cell tumors of the bone, multiple myeloma, osteosarcoma (osteogenic sarcoma), fibrosarcomas and malignant fibrous histiocytomas, chondrosarcomas, Ewing's tumor (Ewing's sarcoma), malignant lymphoma of bone (reticulum cell sarcoma, metastatic tumors of the bone), osteoarthritis, and gout and Pseudogout.

- 20 Examples of disorders of joints and connective tissue are rheumatoid arthritis, psoriatic arthritis, discoid lupus erythematosus, systemic lupus erythematosus, scleroderma (systemic sclerosis), Sjögren's syndrome, connective tissue disease, polymyositis and dermatomyositis, relapsing polychondritis, vasculitis, polyarteritis nodosa, polymyalgia rheumatica, temporal arteritis, Wegener's granulomatosis, Reiter's syndrome, Behcet's syndrome, ankylosing spondylitis, or Charcot's joints (neuropathic joint disease).

Examples for bone and joint infections are osteomyelitis, and infectious arthritis.

Examples of disorders of muscles, bursas, and tendons are spasmodic torticollis, fibromyalgia syndromes (myofascial pain syndromes, fibromyositis), bursitis, tendinitis and tenosynovitis.

Foot problems are, for example ankle sprain, foot fractures, heel spurs, Sever's disease, posterior achilles tendon bursitis, anterior achilles tendon bursitis, posterior tibial neuralgia, pain in the ball of the foot (caused by damage to the nerves between the toes or to the joints between the toes and foot), onychomycosis, or nail discoloration.

5 The human PRKG1 is highly expressed in the following muscle/skeleton tissues: skeletal muscle, cartilage, bone connective tissue, adipose. The expression in muscle/skeleton tissues demonstrates that the human PRKG1 or mRNA can be utilized to diagnose of diseases of the muscle/skeleton system. Additionally the activity of the human PRKG1 can be modulated to treat those diseases.

10 *Cancer Disorders*

Cancer disorders within the scope of this definition comprise any disease of an organ or tissue in mammals characterized by poorly controlled or uncontrolled multiplication of normal or abnormal cells in that tissue and its effect on the body as a whole. Cancer diseases within the scope of the definition comprise benign neoplasms, dysplasias, hyperplasias as well as neoplasms showing metastatic growth or any other transformations like e.g. leukoplakias which often precede a breakout of cancer. Cells and tissues are cancerous when they grow more rapidly than normal cells, displacing or spreading into the surrounding healthy tissue or any other tissues of the body described as metastatic growth, assume abnormal shapes and sizes, show changes in their nucleocytoplasmatic ratio, nuclear polychromasia, and finally may cease. Cancerous cells and tissues may affect the body as a whole when causing paraneoplastic syndromes or if cancer occurs within a vital organ or tissue, normal function will be impaired or halted, with possible fatal results. The ultimate involvement of a vital organ by cancer, either primary or metastatic, may lead to the death of the mammal affected. Cancer tends to spread, and the extent of its spread is usually related to an individual's chances of surviving the disease. Cancers are generally said to be in one of three stages of growth: early, or localized, when a tumor is still confined to the tissue of origin, or primary site; direct extension, where cancer cells from the tumour have invaded adjacent tissue or have spread only to regional lymph nodes; or metastasis, in which cancer cells have migrated to distant parts of the body from the primary site, via the blood or lymph systems, and have established secondary sites of infection. Cancer is said to be malignant because of its tendency to cause death if not treated. Benign tumors usually do not cause death, although they may if they interfere with a normal body function by virtue of their location, size, or paraneoplastic side effects. Hence benign tumors fall under the definition of cancer within the scope of this definition as well. In general, cancer cells divide at a higher rate than do normal cells, but the distinction between the growth of cancerous and normal tissues is not so much the rapidity of cell division in

the former as it is the partial or complete loss of growth restraint in cancer cells and their failure to differentiate into a useful, limited tissue of the type that characterizes the functional equilibrium of growth of normal tissue. Cancer tissues may express certain molecular receptors and probably are influenced by the host's susceptibility and immunity and it is known that certain cancers of the
5 breast and prostate, for example, are considered dependent on specific hormones for their existence. The term "cancer" under the scope of the definition is not limited to simple benign neoplasia but comprises any other benign and malign neoplasia like 1) Carcinoma, 2) Sarcoma, 3) Carcinosarcoma, 4) Cancers of the blood-forming tissues, 5) tumors of nerve tissues including the brain, 6) cancer of skin cells. Cancer according to 1) occurs in epithelial tissues, which cover the
10 outer body (the skin) and line mucous membranes and the inner cavitary structures of organs e.g. such as the breast, lung, the respiratory and gastrointestinal tracts, the endocrine glands, and the genitourinary system. Ductal or glandular elements may persist in epithelial tumors, as in adenocarcinomas like e.g. thyroid adenocarcinoma, gastric adenocarcinoma, uterine adenocarcinoma. Cancers of the pavement-cell epithelium of the skin and of certain mucous membranes,
15 such as e.g. cancers of the tongue, lip, larynx, urinary bladder, uterine cervix, or penis, may be termed epidermoid or squamous-cell carcinomas of the respective tissues and are in the scope of the definition of cancer as well. Cancer according to 2) develops in connective tissues, including fibrous tissues, adipose (fat) tissues, muscle, blood vessels, bone, and cartilage like e.g. osteogenic sarcoma; liposarcoma, fibrosarcoma, synovial sarcoma. Cancer according to 3) is cancer that
20 develops in both epithelial and connective tissue. Cancer disease within the scope of this definition may be primary or secondary, whereby primary indicates that the cancer originated in the tissue where it is found rather than was established as a secondary site through metastasis from another lesion. Cancers and tumor diseases within the scope of this definition may be benign or malign and may affect all anatomical structures of the body of a mammal. By example but not limited to they
25 comprise cancers and tumor diseases of I) the bone marrow and bone marrow derived cells (leukemias), II) the endocrine and exocrine glands like e.g. thyroid, parathyroid, pituitary, adrenal glands, salivary glands, pancreas III) the breast, like e.g. benign or malignant tumors in the mammary glands of either a male or a female, the mammary ducts, adenocarcinoma, medullary carcinoma, comedo carcinoma, Paget's disease of the nipple, inflammatory carcinoma of the young
30 woman, IV) the lung, V) the stomach, VI) the liver and spleen, VII) the small intestine, VIII) the colon, IX) the bone and its supportive and connective tissues like malignant or benign bone tumour, e.g. malignant osteogenic sarcoma, benign osteoma, cartilage tumors; like malignant chondrosarcoma or benign chondroma; bone marrow tumors like malignant myeloma or benign eosinophilic granuloma, as well as metastatic tumors from bone tissues at other locations of the
35 body; X) the mouth, throat, larynx, and the esophagus, XI) the urinary bladder and the internal and external organs and structures of the urogenital system of male and female like ovaries, uterus,

- cervix of the uterus, testes, and prostate gland, XII) the prostate, XIII) the pancreas, like ductal carcinoma of the pancreas; XIV) the lymphatic tissue like lymphomas and other tumors of lymphoid origin, XV) the skin, XVI) cancers and tumor diseases of all anatomical structures belonging to the respiration and respiratory systems including thoracal muscles and linings, XVII)
- 5 primary or secondary cancer of the lymph nodes XVIII) the tongue and of the bony structures of the hard palate or sinuses, XIX) the mouth, cheeks, neck and salivary glands, XX) the blood vessels including the heart and their linings, XXI) the smooth or skeletal muscles and their ligaments and linings, XXII) the peripheral, the autonomous, the central nervous system including the cerebellum, XXIII) the adipose tissue.
- 10 The human PRKG1 is highly expressed in the following cancer tissues: esophagus tumor, stomach tumor, colon tumor, ileum tumor, uterus tumor, ovary tumor, breast tumor, prostate tumor. The expression in the above mentioned tissues and in particular the differential expression between diseased tissue esophagus tumor and healthy tissue esophagus, between diseased tissue stomach tumor and healthy tissue stomach, between diseased tissue colon tumor and healthy tissue colon,
- 15 between diseased tissue ileum tumor and healthy tissue ileum, between diseased tissue uterus tumor and healthy tissue uterus, between diseased tissue ovary tumor and healthy tissue ovary, between diseased tissue breast tumor and healthy tissue breast, between diseased tissue prostate tumor and healthy tissue prostate demonstrates that the human PRKG1 or mRNA can be utilized to diagnose of cancer. Additionally the activity of the human PRKG1 can be modulated to treat
- 20 cancer.

Inflammatory Diseases

Inflammatory diseases comprise diseases triggered by cellular or non-cellular mediators of the immune system or tissues causing the inflammation of body tissues and subsequently producing an acute or chronic inflammatory condition. Examples for such inflammatory diseases are

25 hypersensitivity reactions of type I – IV, for example but not limited to hypersensitivity diseases of the lung including asthma, atopic diseases, allergic rhinitis or conjunctivitis, angioedema of the lids, hereditary angioedema, antireceptor hypersensitivity reactions and autoimmune diseases, Hashimoto's thyroiditis, systemic lupus erythematosus, Goodpasture's syndrome, pemphigus, myasthenia gravis, Grave's and Raynaud's disease, type B insulin-resistant diabetes, rheumatoid

30 arthritis, psoriasis, Crohn's disease, scleroderma, mixed connective tissue disease, polymyositis, sarcoidosis, glomerulonephritis, acute or chronic host versus graft reactions.

The human PRKG1 is highly expressed in the following tissues of the immune system and tissues responsive to components of the immune system as well as in the following tissues responsive to mediators of inflammation: pancreas liver cirrhosis, liver liver cirrhosis, leukocytes (peripheral

blood), bone marrow, spleen liver cirrhosis. The expression in the above mentioned tissues and in particular the differential expression between diseased tissue pancreas liver cirrhosis and healthy tissue pancreas, between diseased tissue liver liver cirrhosis and healthy tissue liver, between diseased tissue spleen liver cirrhosis and healthy tissue spleen demonstrates that the human 5 PRKG1 or mRNA can be utilized to diagnose of inflammatory diseases. Additionally the activity of the human PRKG1 can be modulated to treat inflammatory diseases.

Disorders Related to Pulmology

Asthma is thought to arise as a result of interactions between multiple genetic and environmental factors and is characterized by three major features: 1) intermittent and reversible airway 10 obstruction caused by bronchoconstriction, increased mucus production, and thickening of the walls of the airways that leads to a narrowing of the airways, 2) airway hyperresponsiveness, and 15 3) airway inflammation. Certain cells are critical to the inflammatory reaction of asthma and they include T cells and antigen presenting cells, B cells that produce IgE, and mast cells, basophils, eosinophils, and other cells that bind IgE. These effector cells accumulate at the site of allergic reaction in the airways and release toxic products that contribute to the acute pathology and eventually to tissue destruction related to the disorder. Other resident cells, such as smooth muscle 20 cells, lung epithelial cells, mucus-producing cells, and nerve cells may also be abnormal in individuals with asthma and may contribute to its pathology. While the airway obstruction of asthma, presenting clinically as an intermittent wheeze and shortness of breath, is generally the most pressing symptom of the disease requiring immediate treatment, the inflammation and tissue destruction associated with the disease can lead to irreversible changes that eventually make asthma a chronic and disabling disorder requiring long-term management.

Chronic obstructive pulmonary (or airways) disease (COPD) is a condition defined physiologically as airflow obstruction that generally results from a mixture of emphysema and peripheral airway 25 obstruction due to chronic bronchitis [Botstein, 1980]. Emphysema is characterised by destruction of alveolar walls leading to abnormal enlargement of the air spaces of the lung. Chronic bronchitis is defined clinically as the presence of chronic productive cough for three months in each of two successive years. In COPD, airflow obstruction is usually progressive and is only partially reversible. By far the most important risk factor for development of COPD is cigarette smoking, 30 although the disease does also occur in non-smokers.

The human PRKG1 is highly expressed in the following tissues of the respiratory system: pulmonary artery, pulmonary artery smooth muscle cells, leukocytes (peripheral blood), fetal lung, fetal lung fibroblast IMR-90 cells, lung, lung right upper lobe, lung right mid lobe, lung right lower lobe, trachea, primary bronchia, secondary bronchia, bronchial smooth muscle cells. The

expression in the above mentioned tissues and in particular the differential expression between diseased tissue fetal lung fibroblast IMR-90 cells and healthy tissue fetal lung demonstrates that the human PRKG1 or mRNA can be utilized to diagnose of respiratory diseases. Additionally the activity of the human PRKG1 can be modulated to treat those diseases.

5 *Disorders Related to Urology*

Genitourinary disorders comprise benign and malign disorders of the organs constituting the genitourinary system of female and male, renal diseases like acute or chronic renal failure, immunologically mediated renal diseases like renal transplant rejection, lupus nephritis, immune complex renal diseases, glomerulopathies, nephritis, toxic nephropathy, obstructive uropathies like
10 benign prostatic hyperplasia (BPH), neurogenic bladder syndrome, urinary incontinence like urge-, stress-, or overflow incontinence, pelvic pain, and erectile dysfunction.

The human PRKG1 is highly expressed in the following urological tissues: spinal cord, spinal cord (ventral horn), spinal cord (dorsal horn), prostate, prostate BPH, prostate tumor, bladder, ureter, penis. The expression in the above mentioned tissues and in particular the differential expression
15 between diseased tissue prostate BPH and healthy tissue prostate demonstrates that the human PRKG1 or mRNA can be utilized to diagnose of urological disorders. Additionally the activity of the human PRKG1 can be modulated to treat urological disorders.

The human PRKG1 is highly expressed in spinal cord tissues: spinal cord, spinal cord (ventral horn), spinal cord (dorsal horn). Expression in spinal cord tissues demonstrates that the human
20 PRKG1 or mRNA can be utilized to diagnose of incontinence as an urological disorder. The spinal cord tissues are involved in the neuronal regulation of the urological system. Additionally the activity of the human PRKG1 can be modulated to treat - but not limited to - incontinence.

Metabolic Disorders

Metabolic diseases are defined as conditions which result from an abnormality in any of the
25 chemical or biochemical transformations and their regulating systems essential to producing energy, to regenerating cellular constituents, to eliminating unneeded products arising from these processes, and to regulate and maintain homeostasis in a mammal regardless of whether acquired or the result of a genetic transformation. Depending on which metabolic pathway is involved, a single defective transformation or disturbance of its regulation may produce consequences that are
30 narrow, involving a single body function, or broad, affecting many organs, organ-systems or the body as a whole. Diseases resulting from abnormalities related to the fine and coarse mechanisms that affect each individual transformation, its rate and direction or the availability of substrates like

amino acids, fatty acids, carbohydrates, minerals, cofactors, hormones, regardless whether they are inborn or acquired, are well within the scope of the definition of a metabolic disease according to this application.

Metabolic diseases often are caused by single defects in particular biochemical pathways, defects

5 that are due to the deficient activity of individual enzymes or molecular receptors leading to the regulation of such enzymes. Hence in a broader sense disturbances of the underlying genes, their products and their regulation lie well within the scope of this definition of a metabolic disease. For example, but not limited to, metabolic diseases may affect 1) biochemical processes and tissues ubiquitous all over the body, 2) the bone, 3) the nervous system, 4) the endocrine system, 5) the
10 muscle including the heart, 6) the skin and nervous tissue, 7) the urogenital system, 8) the homeostasis of body systems like water and electrolytes. For example, but not limited to, metabolic diseases according to 1) comprise obesity, amyloidosis, disturbances of the amino acid metabolism like branched chain disease, hyperaminoacidemia, hyperaminoaciduria, disturbances of the metabolism of urea, hyperammonemia, mucopolysaccharidoses e.g. Maroteaux-Lamy
15 syndrom, storage diseases like glycogen storage diseases and lipid storage diseases, glycogenosis diseases like Cori's disease, malabsorption diseases like intestinal carbohydrate malabsorption, oligosaccharidase deficiency like maltase-, lactase-, sucrase-insufficiency, disorders of the metabolism of fructose, disorders of the metabolism of galactose, galactosaemia, disturbances of carbohydrate utilization like diabetes, hypoglycemia, disturbances of pyruvate metabolism,
20 hypolipidemia, hypolipoproteinemia, hyperlipidemia, hyperlipoproteinemia, carnitine or carnitine acyltransferase deficiency, disturbances of the porphyrin metabolism, porphyrias, disturbances of the purine metabolism, lysosomal diseases, metabolic diseases of nerves and nervous systems like gangliosidoses, sphingolipidoses, sulfatidoses, leucodystrophies, Lesch-Nyhan syndrome. For example, but not limited to, metabolic diseases according to 2) comprise osteoporosis,
25 osteomalacia like osteoporosis, osteopenia, osteogenesis imperfecta, osteopetrosis, osteonecrosis, Paget's disease of bone, hypophosphatemia. For example, but not limited to, metabolic diseases according to 3) comprise cerebellar dysfunction, disturbances of brain metabolism like dementia, Alzheimer's disease, Huntington's chorea, Parkinson's disease, Pick's disease, toxic encephalopathy, demyelinating neuropathies like inflammatory neuropathy, Guillain-Barré syndrome. For
30 example, but not limited to, metabolic diseases according to 4) comprise primary and secondary metabolic disorders associated with hormonal defects like any disorder stemming from either an hyperfunction or hypofunction of some hormone-secreting endocrine gland and any combination thereof. They comprise Sipple's syndrome, pituitary gland dysfunction and its effects on other endocrine glands, such as the thyroid, adrenals, ovaries, and testes, acromegaly, hyper- and
35 hypothyroidism, euthyroid goiter, euthyroid sick syndrome, thyroiditis, and thyroid cancer, over-

or underproduction of the adrenal steroid hormones, adrenogenital syndrome, Cushing's syndrome, Addison's disease of the adrenal cortex, Addison's pernicious anemia, primary and secondary aldosteronism, diabetes insipidus, carcinoid syndrome, disturbances caused by the dysfunction of the parathyroid glands, pancreatic islet cell dysfunction, diabetes, disturbances of the endocrine system of the female like estrogen deficiency, resistant ovary syndrome. For example, but not limited to, metabolic diseases according to 5) comprise muscle weakness, myotonia, Duchenne's and other muscular dystrophies, dystrophia myotonica of Steinert, mitochondrial myopathies like disturbances of the catabolic metabolism in the muscle, carbohydrate and lipid storage myopathies, glycogenoses, myoglobinuria, malignant hyperthermia, polymyalgia rheumatica, dermatomyositis, 10 primary myocardial disease, cardiomyopathy. For example, but not limited to, metabolic diseases according to 6) comprise disorders of the ectoderm, neurofibromatosis, scleroderma and polyarteritis, Louis-Bar syndrome, von Hippel-Lindau disease, Sturge-Weber syndrome, tuberous sclerosis, amyloidosis, porphyria. For example, but not limited to, metabolic diseases according to 7) comprise sexual dysfunction of the male and female. For example, but not limited to, metabolic 15 diseases according to 8) comprise confused states and seizures due to inappropriate secretion of antidiuretic hormone from the pituitary gland, Liddle's syndrome, Bartter's syndrome, Fanconi's syndrome, renal electrolyte wasting, diabetes insipidus.

The human PRKG1 is highly expressed in the following metabolic disease related tissues: thyroid, pancreas, pancreas liver cirrhosis, liver, liver liver cirrhosis, spleen liver cirrhosis, cartilage. The 20 expression in the above mentioned tissues and in particular the differential expression between diseased tissue pancreas liver cirrhosis and healthy tissue pancreas, between diseased tissue liver liver cirrhosis and healthy tissue liver, between diseased tissue spleen liver cirrhosis and healthy tissue spleen demonstrates that the human PRKG1 or mRNA can be utilized to diagnose of metabolic diseases. Additionally the activity of the human PRKG1 can be modulated to treat 25 metabolic diseases.

Applications

The present invention provides for both prophylactic and therapeutic methods for cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases. 30

The regulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of PRKG1. An agent that modulates activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring cognate ligand of the polypeptide, a peptide, a peptidomimetic, or any small molecule. In one embodiment, the agent stimulates one

- or more of the biological activities of PRKG1. Examples of such stimulatory agents include the active PRKG1 and nucleic acid molecules encoding a portion of PRKG1. In another embodiment, the agent inhibits one or more of the biological activities of PRKG1. Examples of such inhibitory agents include antisense nucleic acid molecules and antibodies. These regulatory methods can be
- 5 performed in vitro (e.g., by culturing the cell with the agent) or, alternatively, in vivo (e.g., by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by unwanted expression or activity of PRKG1 or a protein in the PRKG1 signaling pathway. In one embodiment, the method involves administering an agent like any agent identified or being identifiable by a screening assay as
- 10 described herein, or combination of such agents that modulate say upregulate or downregulate the expression or activity of PRKG1 or of any protein in the PRKG1 signaling pathway. In another embodiment, the method involves administering a regulator of PRKG1 as therapy to compensate for reduced or undesirably low expression or activity of PRKG1 or a protein in the PRKG1 signaling pathway.
- 15 Stimulation of activity or expression of PRKG1 is desirable in situations in which enzymatic activity or expression is abnormally low and in which increased activity is likely to have a beneficial effect. Conversely, inhibition of enzymatic activity or expression of PRKG1 is desirable in situations in which activity or expression of PRKG1 is abnormally high and in which decreasing its activity is likely to have a beneficial effect.
- 20 This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are hereby incorporated by reference.

Pharmaceutical Compositions

This invention further pertains to novel agents identified by the above-described screening assays
25 and uses thereof for treatments as described herein.

The nucleic acid molecules, polypeptides, and antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, anti-bacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is

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incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

The invention includes pharmaceutical compositions comprising a regulator of PRKG1 expression or activity (and/or a regulator of the activity or expression of a protein in the PRKG1 signaling pathway) as well as methods for preparing such compositions by combining one or more such regulators and a pharmaceutically acceptable carrier. Also within the invention are pharmaceutical compositions comprising a regulator identified using the screening assays of the invention packaged with instructions for use. For regulators that are antagonists of PRKG1 activity or which reduce PRKG1 expression, the instructions would specify use of the pharmaceutical composition for treatment of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases. For regulators that are agonists of PRKG1 activity or increase PRKG1 expression, the instructions would specify use of the pharmaceutical composition for treatment of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases.

An inhibitor of PRKG1 may be produced using methods which are generally known in the art. In particular, purified PRKG1 may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind PRKG1. Antibodies to PRKG1 may also be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies like those which inhibit dimer formation are especially preferred for therapeutic use.

In another embodiment of the invention, the polynucleotides encoding PRKG1, or any fragment or complement thereof, may be used for therapeutic purposes. In one aspect, the complement of the polynucleotide encoding PRKG1 may be used in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding PRKG1. Thus, complementary molecules or fragments may be used to modulate PRKG1 activity, or to achieve regulation of gene function. Such technology is now well known in the art, and sense or antisense oligonucleotides or larger fragments can be designed from various locations along the coding or control regions of sequences encoding PRKG1.

Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population. Methods which are well known to those skilled in the art can be used to construct vectors which will express nucleic acid sequence complementary to the polynucleotides 5 of the gene encoding PRKG1. These techniques are described, for example, in [Scott and Smith (1990)].

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

10 An additional embodiment of the invention relates to the administration of a pharmaceutical composition containing PRKG1 in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of PRKG1, antibodies to PRKG1, and mimetics, agonists, antagonists, or inhibitors of PRKG1. The compositions may be administered alone or in combination with at least one other agent, such as a 15 stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs or hormones.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, 20 intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid 25 or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

30 Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EM™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid

to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, a pharmaceutically acceptable polyol like glycerol, propylene glycol, liquid 5 polyethyleneglycol, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable 10 to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin. Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a polypeptide or antibody) in the required amount in an appropriate solvent with 15 one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields 20 a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or 25 capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed.

Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following 30 ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from a pressurized container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or
5 transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or
10 creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration. For pharmaceutical compositions which include an antagonist of PRKG1 activity, a compound which reduces expression of PRKG1, or a compound which reduces expression or activity of a protein in the PRKG1 signaling pathway or any combination thereof,

- 5 the instructions for administration will specify use of the composition for cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases. For pharmaceutical compositions which include an agonist of PRKG1 activity, a compound which increases expression of PRKG1, or a
10 compound which increases expression or activity of a protein in the PRKG1 signaling pathway or any combination thereof, the instructions for administration will specify use of the composition for cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases.

15 **Diagnostics**

In another embodiment, antibodies which specifically bind PRKG1 may be used for the diagnosis of disorders characterized by the expression of PRKG1, or in assays to monitor patients being treated with PRKG1 or agonists, antagonists, and inhibitors of PRKG1. Antibodies useful for diagnostic purposes may be prepared in the same manner as those described above for

- 20 therapeutics. Diagnostic assays for PRKG1 include methods which utilize the antibody and a label to detect PRKG1 in human body fluids or in extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by covalent or non-covalent joining with a reporter molecule. A wide variety of reporter molecules, several of which are described above, are known in the art and may be used.

- 25 A variety of protocols for measuring PRKG1, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of PRKG1 expression. Normal or standard values for PRKG1 expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, preferably human, with antibody to PRKG1 under conditions suitable for complex formation. The amount of standard complex formation may
30 be quantified by various methods, preferably by photometric means. Quantities of PRKG1 expressed in subject samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding PRKG1 may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences,

complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantitate gene expression in biopsied tissues in which expression of PRKG1 may be correlated with disease. The diagnostic assay may be used to distinguish between absence, presence, and excess expression of PRKG1, and to monitor regulation of PRKG1 levels during therapeutic intervention.

Polynucleotide sequences encoding PRKG1 may be used for the diagnosis of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases associated with expression of PRKG1. The polynucleotide sequences encoding PRKG1 may be used in Southern, Northern, or dot-blot analysis, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and ELISA assays; and in microarrays utilizing fluids or tissues from patient biopsies to detect altered PRKG1 expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding PRKG1 may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences encoding PRKG1 may be labelled by standard methods and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantitated and compared with a standard value. If the amount of signal in the patient sample is significantly altered from that of a comparable control sample, the nucleotide sequences have hybridized with nucleotide sequences in the sample, and the presence of altered levels of nucleotide sequences encoding PRKG1 in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or in monitoring the treatment of an individual patient.

In order to provide a basis for the diagnosis of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastro-enterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases associated with expression of PRKG1, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding PRKG1, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with values from an experiment in which a known amount of a substantially purified polynucleotide is used. Standard values obtained from normal samples may be compared with values obtained from

samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

Determination of a Therapeutically Effective Dose

The determination of a therapeutically effective dose is well within the capability of those skilled
5 in the art. A therapeutically effective dose refers to that amount of active ingredient which increases or decreases PRKG1 activity relative to PRKG1 activity which occurs in the absence of the therapeutically effective dose. For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rabbits, dogs, or pigs. The animal model also can be used to determine the appropriate concentration range and
10 route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

Therapeutic efficacy and toxicity, e.g., ED₅₀ (the dose therapeutically effective in 50% of the population) and LD₅₀ (the dose lethal to 50% of the population), can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The dose ratio of toxic to
15 therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD₅₀/ED₅₀. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage varies within this range
20 depending upon the dosage form employed, sensitivity of the patient, and the route of administration. The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active ingredient or to maintain the desired effect. Factors which can be taken into account include the severity of the disease state, general health of the subject, age, weight, and
25 gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions can be administered every 3 to 4 days, every week, or once every two weeks depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts can vary from 0.1 micrograms to 100,000 micrograms, up to a total dose
30 of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc. If the reagent is a single-chain antibody, polynucleotides encoding

the antibody can be constructed and introduced into a cell either *ex vivo* or *in vivo* using well-established techniques including, but not limited to, transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads, protoplast fusion, viral infection, 5 electroporation, "gene gun", and DEAE- or calcium phosphate-mediated transfection.

If the expression product is mRNA, the reagent is preferably an antisense oligonucleotide or a ribozyme. Polynucleotides which express antisense oligonucleotides or ribozymes can be introduced into cells by a variety of methods, as described above. Preferably, a reagent reduces expression of PRKG1 gene or the activity of PRKG1 by at least about 10, preferably about 50, 10 more preferably about 75, 90, or 100% relative to the absence of the reagent. The effectiveness of the mechanism chosen to decrease the level of expression of PRKG1 gene or the activity of PRKG1 can be assessed using methods well known in the art, such as hybridization of nucleotide probes to PRKG1-specific mRNA, quantitative RT-PCR, immunologic detection of PRKG1, or measurement of PRKG1 activity.

- 15 In any of the embodiments described above, any of the pharmaceutical compositions of the invention can be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy can be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents can act synergistically to effect the treatment or prevention of the various disorders 20 described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects. Any of the therapeutic methods described above can be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.
- 25 Nucleic acid molecules of the invention are those nucleic acid molecules which are contained in a group of nucleic acid molecules consisting of (i) nucleic acid molecules encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 2, (ii) nucleic acid molecules comprising the sequence of SEQ ID NO: 1, (iii) nucleic acid molecules having the sequence of SEQ ID NO: 1, (iv) nucleic acid molecules the complementary strand of which hybridizes under stringent 30 conditions to a nucleic acid molecule of (i), (ii), or (iii); and (v) nucleic acid molecules the sequence of which differs from the sequence of a nucleic acid molecule of (iii) due to the degeneracy of the genetic code, wherein the polypeptide encoded by said nucleic acid molecule has PRKG1 activity.

Polypeptides of the invention are those polypeptides which are contained in a group of polypeptides consisting of (i) polypeptides having the sequence of SEQ ID NO: 2, (ii) polypeptides comprising the sequence of SEQ ID NO: 2, (iii) polypeptides encoded by nucleic acid molecules of the invention and (iv) polypeptides which show at least 99%, 98%, 95%, 90%, or 80% homology 5 with a polypeptide of (i), (ii), or (iii), wherein said purified polypeptide has PRKG1 activity.

An object of the invention is a method of screening for therapeutic agents useful in the treatment of a disease comprised in a group of diseases consisting of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases in a mammal comprising the steps of (i) contacting a test compound with a PRKG1 polypeptide, (ii) detect binding of said test compound to said PRKG1 polypeptide. E.g., compounds that bind to the PRKG1 polypeptide are identified potential therapeutic agents for such a disease.
10

Another object of the invention is a method of screening for therapeutic agents useful in the treatment of a disease comprised in a group of diseases consisting of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases in a mammal comprising the steps of (i) determining the activity of a PRKG1 polypeptide at a certain concentration of a test compound or 15 in the absence of said test compound, (ii) determining the activity of said polypeptide at a different concentration of said test compound. E.g., compounds that lead to a difference in the activity of the PRKG1 polypeptide in (i) and (ii) are identified potential therapeutic agents for such a disease.
20

Another object of the invention is a method of screening for therapeutic agents useful in the treatment of a disease comprised in a group of diseases consisting of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases in a mammal comprising the steps of (i) determining the activity of a PRKG1 polypeptide at a certain concentration of a test compound, (ii) determining the activity of a PRKG1 polypeptide at the presence of a compound known to be a 25 regulator of a PRKG1 polypeptide. E.g., compounds that show similar effects on the activity of the PRKG1 polypeptide in (i) as compared to compounds used in (ii) are identified potential therapeutic agents for such a disease.
30

Other objects of the invention are methods of the above, wherein the step of contacting is in or at the surface of a cell.

Other objects of the invention are methods of the above, wherein the cell is in vitro.

Other objects of the invention are methods of the above, wherein the step of contacting is in a cell-free system.

Other objects of the invention are methods of the above, wherein the polypeptide is coupled to a
5 detectable label.

Other objects of the invention are methods of the above, wherein the compound is coupled to a detectable label.

Other objects of the invention are methods of the above, wherein the test compound displaces a ligand which is first bound to the polypeptide.

10 Other objects of the invention are methods of the above, wherein the polypeptide is attached to a solid support.

Other objects of the invention are methods of the above, wherein the compound is attached to a solid support.

Another object of the invention is a method of screening for therapeutic agents useful in the
15 treatment of a disease comprised in a group of diseases consisting of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases in a mammal comprising the steps of (i) contacting a test compound with a PRKG1 polynucleotide, (ii) detect binding of said test
20 compound to said PRKG1 polynucleotide. Compounds that, e.g., bind to the PRKG1 polynucleotide are potential therapeutic agents for the treatment of such diseases.

Another object of the invention is the method of the above, wherein the nucleic acid molecule is RNA.

Another object of the invention is a method of the above, wherein the contacting step is in or at the
25 surface of a cell.

Another object of the invention is a method of the above, wherein the contacting step is in a cell-free system.

Another object of the invention is a method of the above, wherein the polynucleotide is coupled to a detectable label.

Another object of the invention is a method of the above, wherein the test compound is coupled to a detectable label.

Another object of the invention is a method of diagnosing a disease comprised in a group of diseases consisting of cardiovascular diseases, respiratory diseases, dermatological diseases, 5 endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases in a mammal comprising the steps of (i) determining the amount of a PRKG1 polynucleotide in a sample taken from said mammal, (ii) determining the amount of PRKG1 polynucleotide in healthy and/or diseased mammal. A disease is diagnosed, e.g., if there is a substantial similarity in the 10 amount of PRKG1 polynucleotide in said test mammal as compared to a diseased mammal.

Another object of the invention is a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastro-enterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological 15 diseases and urological diseases in a mammal comprising a therapeutic agent which binds to a PRKG1 polypeptide.

Another object of the invention is a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastro-enterological 20 diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases in a mammal comprising a therapeutic agent which regulates the activity of a PRKG1 polypeptide.

Another object of the invention is a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of cardiovascular diseases, respiratory diseases, 25 dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastro-enterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases in a mammal comprising a therapeutic agent which regulates the activity of a PRKG1 polypeptide, wherein said therapeutic agent is (i) a small molecule, (ii) an RNA molecule, (iii) an antisense oligonucleotide, (iv) a polypeptide, (v) an antibody, or (vi) a 30 ribozyme.

Another object of the invention is a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastro-

enterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases in a mammal comprising a PRKG1 polynucleotide.

Another object of the invention is a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of cardiovascular diseases, respiratory diseases,

5 dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastro-enterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases in a mammal comprising a PRKG1 polypeptide.

Another object of the invention is the use of regulators of a PRKG1 for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases

10 consisting of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases in a mammal.

Another object of the invention is a method for the preparation of a pharmaceutical composition

15 useful for the treatment of a disease comprised in a group of diseases consisting of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases in a mammal comprising the steps of (i) identifying a regulator of PRKG1, (ii) determining whether said regulator ameliorates the
20 symptoms of a disease comprised in a group of diseases consisting of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases in a mammal; and (iii) combining of said regulator with an acceptable pharmaceutical carrier.

25 Another object of the invention is the use of a regulator of PRKG1 for the regulation of PRKG1 activity in a mammal having a disease comprised in a group of diseases consisting of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases.

30 The uses, methods or compositions of the invention are useful for each single disease comprised in a group of diseases consisting of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological

diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases.

The expression of human PRKG1 in respiratory and urological related tissues (as described above) suggests a particular – but not limited to – utilization of PRKG1 for diagnosis and modulation of 5 respiratory diseases and urological disorders. Furthermore the above described expression suggest a – but not limited to – utilization of PRKG1 to cardiovascular diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases

10 The examples below are provided to illustrate the subject invention. These examples are provided by way of illustration and are not included for the purpose of limiting the invention.

Examples

Example 1: Search for homologous sequences in public sequence data bases

The degree of homology can readily be calculated by known methods. Preferred methods to determine homology are designed to give the largest match between the sequences tested.

- 5 Methods to determine homology are codified in publicly available computer programs such as BestFit, BLASTP, BLASTN, and FASTA. The BLAST programs are publicly available from NCBI and other sources in the internet.

For PRKG1 the following hits to known sequences were identified by using the BLAST algorithm

[Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ; Nucleic Acids

- 10 Res 1997 Sep 1; 25(17): 3389-402] and the following set of parameters: matrix = BLOSUM62 and low complexity filter. The following databases were searched: NCBI (non-redundant database) and DERWENT patent database (Geneseq).

The following hits were found:

>AA2001:ABG04718 Abg04718 Novel human diagnostic protein #4709. 2/2002

- 15 Length = 705, Score = 1383 bits (3580), Expect = 0.0, Identities = 686/686 (100%), Positives = 686/686 (100%)

>AA1998:AAW85040 Aaw85040 PKG-green fluorescent protein fusion product. 10/2003

- Length = 932, Score = 1383 bits (3580), Expect = 0.0, Identities = 686/686 (100%), Positives = 20 686/686 (100%)

>AA1998:AAW85039 Aaw85039 Green fluorescent protein-PKG fusion product. 10/2003

Length = 933, Score = 1383 bits (3580), Expect = 0.0, Identities = 686/686 (100%), Positives = 686/686 (100%)

- 25 >emb|CAB69326.1| unnamed protein product [unidentified] emb|CAD35737.1| unnamed protein product [unidentified] gb|AAP00298.1| Sequence 137 from patent US 6518021
Length = 932, Score = 1383 bits (3580), Expect = 0.0, Identities = 686/686 (100%), Positives = 686/686 (100%)

>emb|CAB69325.1| unnamed protein product [unidentified] emb|CAD35736.1| unnamed protein product [unidentified] gb|AAP00297.1| Sequence 135 from patent US 6518021

Length = 933, Score = 1383 bits (3580), Expect = 0.0, Identities = 686/686 (100%), Positives = 686/686 (100%)

5

>ref|NP_006249.1| protein kinase, cGMP-dependent, type I; Protein kinase, cGMP-dependent, regulatory, type I; cGKI; protein kinase, cGMP-dependent, regulatory, type I, beta [Homo sapiens] sp|P14619|KGPB_HUMAN cGMP-dependent protein kinase 1, beta isozyme (cGK 1 beta) (cGKI-beta) pir|S05702 protein kinase (EC 2.7.1.37), cGMP-dependent, type I-beta - human

10 emb|CAA68810.1| unnamed protein product [Homo sapiens] emb|CAB07437.1| cGMP-dependent protein kinase type I beta [Homo sapiens]

Length = 686, Score = 1383 bits (3580), Expect = 0.0, Identities = 686/686 (100%), Positives = 686/686 (100%)

15 >gb|AAQ02512.1| protein kinase, cGMP-dependent, type I [synthetic construct]

Length = 687, Score = 1383 bits (3580), Expect = 0.0, Identities = 686/686 (100%), Positives = 686/686 (100%)

>sp|P21136|KGPB_BOVIN cGMP-dependent protein kinase 1, beta isozyme (CGK 1 beta) (cGKI-

20 beta) emb|CAA70155.1| cGMP kinase type I alpha [Bos taurus]

Length = 686, Score = 1380 bits (3571), Expect = 0.0, Identities = 684/686 (99%), Positives = 685/686 (99%)

>ref|NP_035290.1| protein kinase, cGMP-dependent, type I [Mus musculus]

25 sp|Q9Z0Z0|KGPB_MOUSE cGMP-dependent protein kinase 1, beta isozyme (CGK 1 beta) (cGKI-beta) gb|AAD16044.1| cGMP-dependent protein kinase type Ib [Mus musculus]

Length = 686, Score = 1378 bits (3566), Expect = 0.0, Identities = 683/686 (99%), Positives = 685/686 (99%)

30 >AA2001:ABG04717 Abg04717 Novel human diagnostic protein #4708. 2/2002

Length = 680, Score = 1216 bits (3145), Expect = 0.0, Identities = 616/685 (89%), Positives = 630/685 (91%), Gaps = 19/685 (2%)

>AA2002:AAO18519 Aao18519 Human insulin receptor signaling modifier SEQ ID NO: 60. 10/2002

Length = 671, Score = 1213 bits (3139), Expect = 0.0, Identities = 612/670 (91%), Positives = 624/670 (93%), Gaps = 11/670 (1%)

5

>sp|Q13976|KGPA_HUMAN cGMP-dependent protein kinase 1, alpha isozyme (CGK 1 alpha) (cGKI-alpha) dbj|BAA08297.1| cGMP-dependent protein kinase type I alpha [Homo sapiens] emb|CAB07436.1| cGMP-dependent protein kinase type I alpha [Homo sapiens]

Length = 671, Score = 1213 bits (3139), Expect = 0.0, Identities = 612/670 (91%), Positives = 10 624/670 (93%), Gaps = 11/670 (1%)

>ref|NP_776861.1| protein kinase, cGMP-dependent, type I [Bos taurus]

sp|P00516|KGPA_BOVIN cGMP-dependent protein kinase 1, alpha isozyme (CGK 1 alpha)

(cGKI-alpha) pir||OKBOG protein kinase (EC 2.7.1.37), cGMP-dependent, type I-alpha - bovine

15 emb|CAA34214.1| unnamed protein product [Bos taurus] prf||1511094A cGMP dependent protein kinase I alpha

Length = 671, Score = 1210 bits (3130), Expect = 0.0, Identities = 610/670 (91%), Positives = 623/670 (92%), Gaps = 11/670 (1%)

20

>dbj|BAC39087.1| unnamed protein product [Mus musculus]

Length = 671, Score = 1208 bits (3125), Expect = 0.0, Identities = 609/670 (90%), Positives = 623/670 (92%), Gaps = 11/670 (1%)

>sp|O77676|KGPA_RABIT cGMP-dependent protein kinase 1, alpha isozyme (CGK 1 alpha)

25 (cGKI-alpha) gb|AAC31192.1| cGMP-dependent protein kinase type 1 alpha [Oryctolagus cuniculus]

Length = 671, Score = 1200 bits (3104), Expect = 0.0, Identities = 605/670 (90%), Positives = 618/670 (92%), Gaps = 11/670 (1%)

30

>gb|AAH54581.1| Unknown (protein for MGC:63955) [Danio rerio]

Length = 667, Score = 1114 bits (2882), Expect = 0.0, Identities = 558/670 (83%), Positives = 600/670 (89%), Gaps = 15/670 (2%)

>gb|AAL76255.1| PKG-Ib [Bombyx mori] gb|AAL76256.1| PKG-Ia [Bombyx mori]

35 Length = 744, Score = 887 bits (2291), Expect = 0.0, Identities = 431/665 (64%), Positives = 532/665 (80%), Gaps = 12/665 (1%)

>gb|AAL76257.1| PKG-II [Bombyx mori]

Length = 738, Score = 858 bits (2216), Expect = 0.0, Identities = 418/649 (64%), Positives = 519/649 (79%), Gaps = 12/649 (1%)

5

>AA2001:ABB67313 Abb67313 Drosophila melanogaster polypeptide SEQ ID NO 28731. 3/2002
Length = 742, Score = 843 bits (2178), Expect = 0.0, Identities = 419/702 (59%), Positives = 529/702 (75%), Gaps = 35/702 (4%)

10 >AA2001:ABB67108 Abb67108 Drosophila melanogaster polypeptide SEQ IDNO 28116. 3/2002
Length = 1088, Score = 843 bits (2178), Expect = 0.0, Identities = 419/702 (59%), Positives = 529/702 (75%), Gaps = 35/702 (4%)

15 >ref|NP_477488.1| foraging CG10033-PB [Drosophila melanogaster] gb|AAG22251.1| CG10033-
PB [Drosophila melanogaster]
Length = 742, Score = 843 bits (2178), Expect = 0.0, Identities = 419/702 (59%), Positives = 529/702 (75%), Gaps = 35/702 (4%)

20 >ref|NP_477487.1| foraging CG10033-PA [Drosophila melanogaster] gb|AAF51082.2| CG10033-
PA [Drosophila melanogaster] gb|AAL29062.1| LD46758p [Drosophila melanogaster]
Length = 1088, Score = 843 bits (2178), Expect = 0.0, Identities = 419/702 (59%), Positives = 529/702 (75%), Gaps = 35/702 (4%)

25 >sp|Q03043|KGP2_DROME cGMP-dependent protein kinase, isozyme 2 forms cD4/T1/T3A/T3B
(CGK)(Foraging protein) pir||B34106 protein kinase (EC 2.7.1.37), cGMP-dependent 2, type 1
[similarity] -fruit fly (Drosophila melanogaster) gb|AAA28455.1| cGMP-dependent protein kinase
Length = 1088, Score = 840 bits (2171), Expect = 0.0, Identities = 418/702 (59%), Positives = 528/702 (75%), Gaps = 35/702 (4%)

30 >gb|AAA28459.1| cGMP-dependent protein kinase
Length = 742, Score = 840 bits (2171), Expect = 0.0, Identities = 418/702 (59%), Positives = 528/702 (75%), Gaps = 35/702 (4%)

35 >gb|AAL93136.1| cGMP-dependent protein kinase foraging [Apis mellifera]
Length = 678, Score = 822 bits (2124), Expect = 0.0, Identities = 417/694 (60%), Positives = 515/694 (74%), Gaps = 24/694 (3%)

>gb|AAR06171.1| PKG [Aplysia californica]

Length = 733, Score = 762 bits (1968), Expect = 0.0, Identities = 400/704 (56%), Positives = 506/704 (71%), Gaps = 37/704 (5%)

5

>ref|XP_319605.1| ENSANGP00000006403 [Anopheles gambiae] gb|EAA14900.2|
ENSANGP00000006403 [Anopheles gambiae str. PEST]

Length = 887, Score = 749 bits (1935), Expect = 0.0, Identities = 359/540 (66%), Positives = 438/540 (81%), Gaps = 4/540 (0%)

10

>AA2001:ABB67315 Abb67315 Drosophila melanogaster polypeptide SEQ ID NO 28737. 3/2002
Length = 934, Score = 733 bits (1893), Expect = 0.0, Identities = 354/576 (61%), Positives = 446/576 (77%), Gaps = 19/576 (3%)

15

>AA2001:ABB67314 Abb67314 Drosophila melanogaster polypeptide SEQ ID NO 28734. 3/2002
Length = 934, Score = 733 bits (1893), Expect = 0.0, Identities = 354/576 (61%), Positives = 446/576 (77%), Gaps = 19/576 (3%)

20

>ref|NP_477490.1| foraging CG10033-PE [Drosophila melanogaster] gb|AAG22254.1| CG10033-
PE [Drosophila melanogaster]
Length = 934, Score = 733 bits (1893), Expect = 0.0, Identities = 354/576 (61%), Positives = 446/576 (77%), Gaps = 19/576 (3%)

25

>pir|T08418 protein kinase (EC 2.7.1.37), cGMP-dependent, type cD5 - fruit fly (Drosophila
melanogaster) gb|AAA28454.1| cGMP-dependent protein kinase
Length = 934, Score = 733 bits (1893), Expect = 0.0, Identities = 352/576 (61%), Positives = 446/576 (77%), Gaps = 19/576 (3%)

30

>AA2001:ABB67316 Abb67316 Drosophila melanogaster polypeptide SEQ ID NO 28740. 3/2002
Length = 894, Score = 733 bits (1891), Expect = 0.0, Identities = 347/530 (65%), Positives = 432/530 (81%), Gaps = 4/530 (0%)

35

>gb|AAA28457.1| cGMP-dependent protein kinase
Length = 894, Score = 733 bits (1891), Expect = 0.0, Identities = 347/530 (65%), Positives = 432/530 (81%), Gaps = 4/530 (0%)

>sp|P32023|KGP3_DROME cGMP-dependent protein kinase, isozyme 2 forms cD5/T2 (CGK) (Foraging protein)

Length = 934, Score = 733 bits (1891), Expect = 0.0, Identities = 352/576 (61%), Positives = 446/576 (77%), Gaps = 19/576 (3%)

5

>ref|NP_477489.1| foraging CG10033-PC [Drosophila melanogaster] ref|NP_599146.1| foraging CG10033-PD [Drosophila melanogaster] gb|AAG22253.1| CG10033-PD [Drosophila melanogaster] gb|AAG22252.2| CG10033-PC [Drosophila melanogaster] gb|AAO45237.1| GH10421p [Drosophila melanogaster] gb|AAD34763.2| LD21570p [Drosophila melanogaster]

10 Length = 894, Score = 733 bits (1891), Expect = 0.0, Identities = 347/530 (65%), Positives = 432/530 (81%), Gaps = 4/530 (0%)

>gb|AAA28456.1| cGMP-dependent protein kinase gb|AAA28458.1| cGMP-dependent protein kinase

15 Length = 894, Score = 731 bits (1886), Expect = 0.0, Identities = 346/530 (65%), Positives = 432/530 (81%), Gaps = 4/530 (0%)

>ref|NP_500142.1| EGg Laying defective EGL-4, ODoRant response abnormal ODR-9, Cyclic GMP-dependent Kinase (egl-4) [Caenorhabditis elegans] gb|AAD36953.1| Egg laying defective protein 4, isoform b [Caenorhabditis elegans]

20 Length = 737, Score = 697 bits (1800), Expect = 0.0, Identities = 354/714 (49%), Positives = 479/714 (67%), Gaps = 39/714 (5%)

>ref|NP_741329.1| EGg Laying defective EGL-4, ODoRant response abnormal ODR-9, Cyclic GMP-dependent Kinase (84.5 kD) (egl-4) [Caenorhabditis elegans] gb|AAM98010.1| Egg laying defective protein 4, isoform c [Caenorhabditis elegans]

Length = 749, Score = 697 bits (1800), Expect = 0.0, Identities = 354/714 (49%), Positives = 479/714 (67%), Gaps = 39/714 (5%)

30 >ref|NP_500141.1| EGg Laying defective EGL-4, ODoRant response abnormal ODR-9, Cyclic GMP-dependent Kinase (86.7 kD) (egl-4) [Caenorhabditis elegans] gb|AAD36954.1| Egg laying defective protein 4, isoform a [Caenorhabditis elegans]

Length = 780, Score = 697 bits (1800), Expect = 0.0, Identities = 354/714 (49%), Positives = 479/714 (67%), Gaps = 39/714 (5%)

35

>AA2001:ABB59753 Abb59753 Drosophila melanogaster polypeptide SEQ ID NO 6051. 3/2002
Length = 768, Score = 695 bits (1794), Expect = 0.0, Identities = 350/628 (55%), Positives =
451/628 (71%), Gaps = 17/628 (2%)

- 5 >ref|NP_477213.1| cGMP-dependent protein kinase 21D CG3324-PA [Drosophila melanogaster]
sp|Q03042|KGP1_DROME cGMP-dependent protein kinase, isozyme 1 (CGK) pir|A34106
protein kinase (EC 2.7.1.37), cGMP-dependent 1 - fruit fly (Drosophila melanogaster)
gb|AAA28453.1| cGMP-dependent protein kinase gb|AAF51459.1| CG3324-PA [Drosophila
melanogaster] gb|AAL13517.1| GH03852p [Drosophila melanogaster]
- 10 Length = 768, Score = 695 bits (1794), Expect = 0.0, Identities = 350/628 (55%), Positives =
451/628 (71%), Gaps = 17/628 (2%)

- >gb|AAB03405.1| cGMP-dependent protein kinase [Drosophila melanogaster]
Length = 768, Score = 694 bits (1790), Expect = 0.0, Identities = 350/628 (55%), Positives =
15 449/628 (71%), Gaps = 17/628 (2%)

- >ref|XP_314690.1| ENSANGP00000013014 [Anopheles gambiae] gb|EAA10189.1|
ENSANGP00000013014 [Anopheles gambiae str. PEST]
Length = 656, Score = 692 bits (1786), Expect = 0.0, Identities = 347/624 (55%), Positives =
20 452/624 (72%), Gaps = 13/624 (2%)

- >emb|CAE63845.1| Hypothetical protein CBG08401 [Caenorhabditis briggsae]
Length = 777, Score = 689 bits (1779), Expect = 0.0, Identities = 351/714 (49%), Positives =
479/714 (67%), Gaps = 39/714 (5%)
- 25 >gb|AAC23588.1| cyclic GMP-dependent protein kinase [Hydra oligactis]
Length = 742, Score = 683 bits (1762), Expect = 0.0, Identities = 353/715 (49%), Positives =
486/715 (67%), Gaps = 51/715 (7%)

- 30 >dbj|BAC30119.1| unnamed protein product [Mus musculus]
Length = 762, Score = 676 bits (1743), Expect = 0.0, Identities = 353/728 (48%), Positives =
479/728 (65%), Gaps = 54/728 (7%)

>ref|NP_006250.1| protein kinase, cGMP-dependent, type II; cGKII [Homo sapiens] sp|Q13237|KGP2_HUMAN cGMP-dependent protein kinase 2 (CGK 2) (cGKII) (Type II cGMP-dependent protein kinase) emb|CAA64318.1| Type II cGMP-dependent protein kinase [Homo sapiens]

5 Length = 762, Score = 676 bits (1743), Expect = 0.0, Identities = 354/733 (48%), Positives = 478/733 (65%), Gaps = 54/733 (7%).

Example 2: Expression profiling

10 Total cellular RNA was isolated from cells by one of two standard methods: 1) guanidine isothiocyanate/Cesium chloride density gradient centrifugation [Kellogg, (1990)]; or with the Tri-Reagent protocol according to the manufacturer's specifications (Molecular Research Center, Inc., Cincinnati, Ohio). Total RNA prepared by the Tri-reagent protocol was treated with DNase I to remove genomic DNA contamination.

15 For relative quantitation of the mRNA distribution of PRKG1, total RNA from each cell or tissue source was first reverse transcribed. 85 µg of total RNA was reverse transcribed using 1 µmole random hexamer primers, 0.5 mM each of dATP, dCTP, dGTP and dTTP (Qiagen, Hilden, Germany), 3000 U RnaseQut (Invitrogen, Groningen, Netherlands) in a final volume of 680 µl. The first strand synthesis buffer and Omniscript reverse transcriptase (2 u/µl) were from (Qiagen, Hilden, Germany). The reaction was incubated at 37°C for 90 minutes and cooled on ice. The 20 volume was adjusted to 6800 µl with water, yielding a final concentration of 12.5 ng/µl of starting RNA.

25 For relative quantitation of the distribution of PRKG1 mRNA in cells and tissues the Perkin Elmer ABI Prism RTM. 7700 Sequence Detection system or Biorad iCycler was used according to the manufacturer's specifications and protocols. PCR reactions were set up to quantitate PRKG1 and the housekeeping genes HPRT (hypoxanthine phosphoribosyltransferase), GAPDH (glyceraldehyde-3-phosphate dehydrogenase), β-actin, and others. Forward and reverse primers and probes for PRKG1 were designed using the Perkin Elmer ABI Primer Express™ software and were synthesized by TibMolBiol (Berlin, Germany). The PRKG1 forward primer sequence was: Primer1 (SEQ ID NO: 3). The PRKG1 reverse primer sequence was Primer2 (SEQ ID NO: 4).
30 Probe1 (SEQ ID NO: 5), labelled with FAM (carboxyfluorescein succinimidyl ester) as the reporter dye and TAMRA (carboxytetramethylrhodamine) as the quencher, is used as a probe for PRKG1. The following reagents were prepared in a total of 25 µl : 1x TaqMan buffer A, 5.5 mM MgCl₂, 200 nM of dATP, dCTP, dGTP, and dUTP, 0.025 U/µl AmpliTaq Gold™, 0.01 U/µl AmpErase and Probe1 (SEQ ID NO: 5), PRKG1 forward and reverse primers each at 200 nM,

200 nM PRKG1 FAM/TAMRA-labelled probe, and 5 µl of template cDNA. Thermal cycling parameters were 2 min at 50°C, followed by 10 min at 95°C, followed by 40 cycles of melting at 95°C for 15 sec and annealing/extending at 60°C for 1 min.

Calculation of corrected CT values

5 The CT (threshold cycle) value is calculated as described in the "Quantitative determination of nucleic acids" section. The CF-value (factor for threshold cycle correction) is calculated as follows :

1. PCR reactions were set up to quantitate the housekeeping genes (HKG) for each cDNA sample.
- 10 2. CT_{HKG} -values (threshold cycle for housekeeping gene) were calculated as described in the "Quantitative determination of nucleic acids" section.
3. CT_{HKG} -mean values (CT mean value of all HKG tested on one cDNAs) of all HKG for each cDNA are calculated (n = number of HKG):

$$CT_{HKG-n}\text{-mean value} = (CT_{HKG1}\text{-value} + CT_{HKG2}\text{-value} + \dots + CT_{HKG-n}\text{-value}) / n$$

- 15 4. CT_{panel} mean value (CT mean value of all HKG in all tested cDNAs) =
 $(CT_{HKG1}\text{-mean value} + CT_{HKG2}\text{-mean value} + \dots + CT_{HKG-y}\text{-mean value}) / y$
(y = number of cDNAs)
5. CF_{cDNA-n} (correction factor for cDNA n) = $CT_{panel}\text{-mean value} - CT_{HKG-n}\text{-mean value}$
6. CT_{cDNA-n} (CT value of the tested gene for the cDNA n) + CF_{cDNA-n} (correction factor for cDNA n) = $CT_{cor-cDNA-n}$ (corrected CT value for a gene on cDNA n)

Calculation of relative expression

Definition : highest $CT_{cor-cDNA-n} \neq 40$ is defined as $CT_{cor-cDNA}$ [high]

Relative Expression = $2^{(CT_{cor-cDNA[\text{high}]} - CT_{cor-cDNA-n})}$

Tissues

25 The expression of PRKG1 was investigated in the tissues in table 1.

Expression profile

The results of the mRNA-quantification (expression profiling) is shown in Table 1.

Table 1: *Relative expression of PRKG1 in various human tissues*

fetal heart	127
heart	3169
pericardium	1885
heart atrium (right)	1017
heart atrium (left)	24154
heart ventricle (left)	8719
heart ventricle (right)	92042
heart apex	2226
Purkinje fibers	3397
interventricular septum	1563
fetal aorta	484
aorta	65083
aorta sclerotic	3902
artery	491
coronary artery	274
coronary artery sclerotic	122
pulmonary artery	832
carotid artery	399
mesenteric artery	6794
vein	491
pulmonic valve	1479
coronary artery endothel cells	10809
coronary artery smooth muscle primary cells	2165
aortic smooth muscle cells	744
pulmonary artery smooth muscle cells	234
aortic endothel cells	505
HUVEC cells	17
pulmonary artery endothel cells	66
iliac artery endothel cells	0
skin	41476
adrenal gland	21321
thyroid	152664

thyroid tumor	288
pancreas	3616
pancreas liver cirrhosis	27364
esophagus	48645
esophagus tumor	399
stomach	14462
stomach tumor	1053
colon	21619
colon tumor	101
small intestine	9675
ileum	3468
ileum tumor	69
ileum chronic inflammation	0
rectum	70240
salivary gland	11268
fetal liver	194
liver	474
liver liver cirrhosis	338783
liver lupus disease	207
liver tumor	193
HEP G2 cells	0
leukocytes (peripheral blood)	2998
Jurkat (T-cells)	8
bone marrow	3822
erythrocytes	803
lymphnode	37
thymus	288
thrombocytes	181
bone marrow stromal cells	8
bone marrow CD71+ cells	44
bone marrow CD33+ cells	0
bone marrow CD34+ cells	1965
bone marrow CD15+ cells	6
cord blood CD71+ cells	0

cord blood CD34+ cells	568
neutrophils cord blood	0
neutrophils peripheral blood	0
spleen	734
spleen liver cirrhosis	47644
skeletal muscle	31216
cartilage	4360
bone connective tissue	7968
adipose	771
fetal brain	1031
brain	4211
Alzheimer brain	67378
cerebellum	25355
Alzheimer brain cerebellum	605
cerebellum (right)	307
cerebellum (left)	1024
cerebral cortex	734
Alzheimer cerebral cortex	204253
frontal lobe	1438
Alzheimer brain frontal lobe	534
occipital lobe	177813
parietal lobe	164759
temporal lobe	107204
postcentral gyrus	5713
vermis cerebelli	781
pons	360
substantia nigra	936
caudatum	891
cerebral meninges	189
cerebral peduncles	55878
corpus callosum	635
nucleus accumbens	64634
putamen	52499
hippocampus	111757

thalamus	36611
posteroventral thalamus	81811
dorsalmedial thalamus	55878
hypothalamus	335
dorsal root ganglia	116
spinal cord	79573
spinal cord (ventral horn)	90775
spinal cord (dorsal horn)	24834
neuroblastoma SK-N-MC cells	2
neuroblastoma SH-SY5Y cells	59064
neuroblastoma IMR32 cells	2034
glial tumor H4 cells	4705
HEK CNS + APP	2288
neural progenitor cells	942
astrocytes	4973
retina	2665
fetal lung	82380
fetal lung fibroblast IMR-90 cells	488
fetal lung fibroblast MRC-5 cells	861
lung	3616
lung right upper lobe	80684
lung right mid lobe	96618
lung right lower lobe	113317
lung lupus disease	1380
lung tumor	108
lung COPD	3327
trachea	4330
primary bronchia	134757
secondary bronchia	106464
bronchial epithelial cells	695
bronchial smooth muscle cells	42055
small airway epithelial cells	4
cervix	111757
testis	6039

HeLa cells (cervix tumor)	1
placenta	568
uterus	2180
uterus tumor	193
ovary	175365
ovary tumor	111
breast	252
breast tumor	13308
MDA MB 231 cells (breast tumor)	141
mammary gland	1193
prostate	5405
prostate BPH	3566
prostate tumor	165905
bladder	179050
ureter	58251
penis	27364
corpus cavernosum	1184
fetal kidney	1746
kidney	1278
kidney tumor	208
renal epithelial cells	7
HEK 293 cells	765

Example 3: Antisense Analysis

Knowledge of the correct, complete cDNA sequence coding for PRKG1 enables its use as a tool for antisense technology in the investigation of gene function. Oligonucleotides, cDNA or genomic fragments comprising the antisense strand of a polynucleotide coding for PRKG1 are used either *in vitro* or *in vivo* to inhibit translation of the mRNA. Such technology is now well known in the art, and antisense molecules can be designed at various locations along the nucleotide sequences. By treatment of cells or whole test animals with such antisense sequences, the gene of interest is effectively turned off. Frequently, the function of the gene is ascertained by observing behavior at the intracellular, cellular, tissue or organismal level (e.g., lethality, loss of differentiated function, changes in morphology, etc.).

In addition to using sequences constructed to interrupt transcription of a particular open reading frame, modifications of gene expression is obtained by designing antisense sequences to intron regions, promoter/enhancer elements, or even to trans-acting regulatory genes.

Example 4: Expression of PRKG1

5 Expression of PRKG1 is accomplished by subcloning the cDNAs into appropriate expression vectors and transfecting the vectors into expression hosts such as, e.g., *E. coli*. In a particular case, the vector is engineered such that it contains a promoter for β-galactosidase, upstream of the cloning site, followed by sequence containing the amino-terminal Methionine and the subsequent seven residues of β-galactosidase. Immediately following these eight residues is an engineered
10 bacteriophage promoter useful for artificial priming and transcription and for providing a number of unique endonuclease restriction sites for cloning.

Induction of the isolated, transfected bacterial strain with Isopropyl-β-D-thiogalactopyranoside (IPTG) using standard methods produces a fusion protein corresponding to the first seven residues of β-galactosidase, about 15 residues of “linker”, and the peptide encoded within the cDNA. Since
15 cDNA clone inserts are generated by an essentially random process, there is probability of 33% that the included cDNA will lie in the correct reading frame for proper translation. If the cDNA is not in the proper reading frame, it is obtained by deletion or insertion of the appropriate number of bases using well known methods including in vitro mutagenesis, digestion with exonuclease III or mung bean nuclease, or the inclusion of an oligonucleotide linker of appropriate length.

20 The PRKG1 cDNA is shuttled into other vectors known to be useful for expression of proteins in specific hosts. Oligonucleotide primers containing cloning sites as well as a segment of DNA (about 25 bases) sufficient to hybridize to stretches at both ends of the target cDNA is synthesized chemically by standard methods. These primers are then used to amplify the desired gene segment by PCR. The resulting gene segment is digested with appropriate restriction enzymes under
25 standard conditions and isolated by gel electrophoresis. Alternately, similar gene segments are produced by digestion of the cDNA with appropriate restriction enzymes. Using appropriate primers, segments of coding sequence from more than one gene are ligated together and cloned in appropriate vectors. It is possible to optimize expression by construction of such chimeric sequences.

30 Suitable expression hosts for such chimeric molecules include, but are not limited to, mammalian cells such as Chinese Hamster Ovary (CHO) and human 293 cells., insect cells such as Sf9 cells, yeast cells such as *Saccharomyces cerevisiae* and bacterial cells such as *E. coli*. For each of these cell systems, a useful expression vector also includes an origin of replication to allow propagation

in bacteria, and a selectable marker such as the β -lactamase antibiotic resistance gene to allow plasmid selection in bacteria. In addition, the vector may include a second selectable marker such as the neomycin phosphotransferase gene to allow selection in transfected eukaryotic host cells. Vectors for use in eukaryotic expression hosts require RNA processing elements such as 3' polyadenylation sequences if such are not part of the cDNA of interest.

Additionally, the vector contains promoters or enhancers which increase gene expression. Such promoters are host specific and include MMTV, SV40, and metallothioneine promoters for CHO cells; trp, lac, tac and T7 promoters for bacterial hosts; and alpha factor, alcohol oxidase and PGH promoters for yeast. Transcription enhancers, such as the rous sarcoma virus enhancer, are used in mammalian host cells. Once homogeneous cultures of recombinant cells are obtained through standard culture methods, large quantities of recombinantly produced PRKG1 are recovered from the conditioned medium and analyzed using chromatographic methods known in the art. For example, PRKG1 can be cloned into the expression vector pcDNA3, as exemplified herein. This product can be used to transform, for example, HEK293 or COS by methodology standard in the art. Specifically, for example, using Lipofectamine (Gibco BRL catalog no. 18324-020) mediated gene transfer.

Example 5: Isolation of Recombinant PRKG1

PRKG1 is expressed as a chimeric protein with one or more additional polypeptide domains added to facilitate protein purification. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals [Appa Rao, 1997] and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Washington). The inclusion of a cleavable linker sequence such as Factor Xa or enterokinase (Invitrogen, Groningen, The Netherlands) between the purification domain and the PRKG1 sequence is useful to facilitate expression of PRKG1.

25 The following example provides a method for purifying PRKG1.

PRKG1 is generated using the baculovirus expression system BAC-TO-BAC (GIBCO BRL) based on *Autographa californica* nuclear polyhedrosis virus (AcNPV) infection of *Spodoptera frugiperda* insect cells (Sf9 cells).

cDNA encoding kinases cloned into either the donor plasmid pFASTBAC1 or pFASTBAC-HT which contain a mini-Tn7 transposition element. The recombinant plasmid is transformed into DH10BAC competent cells which contain the parent bacmid bMON14272 (AcNPV infectious DNA) and a helper plasmid. The mini-Tn7 element on the pFASTBAC donor can transpose to the

attTn7 attachment site on the bacmid thus introducing the kinase gene into the viral genome. Colonies containing recombinant bacmids are identified by disruption of the *lacZ* gene. The kinase/bacmid construct can then be isolated and infected into insect cells (Sf9 cells) resulting in the production of infectious recombinant baculovirus particles and expression of either unfused
5 recombinant enzyme (pFastbac1) or PRKG1-His fusion protein (pFastbacHT).

Cells are harvested and extracts prepared 24, 48 and 72 hours after transfection. Expression of PRKG1 is confirmed by coomassie staining after sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting onto a PVDF membrane of an unstained SDS-PAGE. The kinase-His fusion protein is detected due to the interaction between the Ni-NTA HRP
10 conjugate and the His-tag which is fused to PRKG1.

Example 6: Production of PRKG1 Specific Antibodies

Two approaches are utilized to raise antibodies to PRKG1, and each approach is useful for generating either polyclonal or monoclonal antibodies. In one approach, denatured protein from reverse phase HPLC separation is obtained in quantities up to 75 mg. This denatured protein is
15 used to immunize mice or rabbits using standard protocols; about 100 µg are adequate for immunization of a mouse, while up to 1 mg might be used to immunize a rabbit. For identifying mouse hybridomas, the denatured protein is radioiodinated and used to screen potential murine B-cell hybridomas for those which produce antibody. This procedure requires only small quantities of protein, such that 20 mg is sufficient for labeling and screening of several thousand clones.

20 In the second approach, the amino acid sequence of an appropriate PRKG1 domain, as deduced from translation of the cDNA, is analyzed to determine regions of high antigenicity. Oligopeptides comprising appropriate hydrophilic regions are synthesized and used in suitable immunization protocols to raise antibodies. The optimal amino acid sequences for immunization are usually at the C-terminus, the N-terminus and those intervening, hydrophilic regions of the polypeptide
25 which are likely to be exposed to the external environment when the protein is in its natural conformation.

Typically, selected peptides, about 15 residues in length, are synthesized using an Applied Biosystems Peptide Synthesizer Model 431A using fmoc-chemistry and coupled to keyhole limpet hemocyanin (KLH; Sigma, St. Louis, MO) by reaction with M-maleimidobenzoyl-N-hydroxy-
30 succinimide ester, MBS. If necessary, a cysteine is introduced at the N-terminus of the peptide to permit coupling to KLH. Rabbits are immunized with the peptide-KLH complex in complete Freund's adjuvant. The resulting antisera are tested for antipeptide activity by binding the peptide

to plastic, blocking with 1% bovine serum albumin, reacting with antisera, washing and reacting with labeled (radioactive or fluorescent), affinity purified, specific goat anti-rabbit IgG.

Hybridomas are prepared and screened using standard techniques. Hybridomas of interest are detected by screening with labeled PRKG1 to identify those fusions producing the monoclonal antibody with the desired specificity. In a typical protocol, wells of plates (FAST; Becton-Dickinson, Palo Alto, CA) are coated during incubation with affinity purified, specific rabbit anti-mouse (or suitable antispecies 1 g) antibodies at 10 mg/ml. The coated wells are blocked with 1% bovine serum albumin, (BSA), washed and incubated with supernatants from hybridomas. After washing the wells are incubated with labeled PRKG1 at 1 mg/ml. Supernatants with specific antibodies bind more labeled PRKG1 than is detectable in the background. Then clones producing specific antibodies are expanded and subjected to two cycles of cloning at limiting dilution. Cloned hybridomas are injected into pristane-treated mice to produce ascites, and monoclonal antibody is purified from mouse ascitic fluid by affinity chromatography on Protein A. Monoclonal antibodies with affinities of at least

15 10^8 M^{-1} , preferably 10^9 to 10^{10} M^{-1} or stronger, are typically made by standard procedures.

Example 7: Diagnostic Test Using PRKG1 Specific Antibodies

Particular PRKG1 antibodies are useful for investigating signal transduction and the diagnosis of infectious or hereditary conditions which are characterized by differences in the amount or distribution of PRKG1 or downstream products of an active signaling cascade.

20 Diagnostic tests for PRKG1 include methods utilizing antibody and a label to detect PRKG1 in human body fluids, membranes, cells, tissues or extracts of such. The polypeptides and antibodies of the present invention are used with or without modification. Frequently, the polypeptides and antibodies are labeled by joining them, either covalently or noncovalently, with a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known
25 and have been reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent agents, chemiluminescent agents, chromogenic agents, magnetic particles and the like.

A variety of protocols for measuring soluble or membrane-bound PRKG1, using either polyclonal or monoclonal antibodies specific for the protein, are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). A two-site monoclonal-based immunoassay utilizing monoclonal antibodies

reactive to two non-interfering epitopes on PRKG1 is preferred, but a competitive binding assay may be employed.

Example 8: Purification of Native PRKG1 Using Specific Antibodies

Native or recombinant PRKG1 is purified by immunoaffinity chromatography using antibodies specific for PRKG1. In general, an immunoaffinity column is constructed by covalently coupling the anti-TRH antibody to an activated chromatographic resin.

Polyclonal immunoglobulins are prepared from immune sera either by precipitation with ammonium sulfate or by purification on immobilized Protein A (Pharmacia LKB Biotechnology, Piscataway N.J.). Likewise, monoclonal antibodies are prepared from mouse ascites fluid by ammonium sulfate precipitation or chromatography on immobilized Protein A. Partially purified immunoglobulin is covalently attached to a chromatographic resin such as CnBr-activated Sepharose (Pharmacia LKB Biotechnology). The antibody is coupled to the resin, the resin is blocked, and the derivative resin is washed according to the manufacturer's instructions.

Such immunoaffinity columns are utilized in the purification of PRKG1 by preparing a fraction from cells containing PRKG1 in a soluble form. This preparation is derived by solubilization of whole cells or of a subcellular fraction obtained via differential centrifugation (with or without addition of detergent) or by other methods well known in the art. Alternatively, soluble PRKG1 containing a signal sequence is secreted in useful quantity into the medium in which the cells are grown.

A soluble PRKG1-containing preparation is passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of PRKG1 (e.g., high ionic strength buffers in the presence of detergent). Then, the column is eluted under conditions that disrupt antibody/protein binding (e.g., a buffer of pH 2-3 or a high concentration of a chaotrope such as urea or thiocyanate ion), and PRKG1 is collected.

25 Example 9: Drug Screening

This invention is particularly useful for screening therapeutic compounds by using PRKG1 or fragments thereof in any of a variety of drug screening techniques.

The following example provides a system for drug screening measuring the kinase activity.

The recombinant kinase-His fusion protein can be purified from the crude lysate by metal-affinity chromatography using Ni-NTA agarose. This allows the specific retention of the recombinant

material (since this is fused to the His-tag) whilst the endogenous insect proteins are washed off. The recombinant material is then eluted by competition with imidazol.

The activity of PRKG1 molecules of the present invention can be measured using a variety of assays that measure PRKG1 activity. For example, PRKG1 enzyme activity can be assessed by a 5 standard *in vitro* kinase assay.

The kinase activity of the kinase can be detected, for example, by adding ATP having radioactively labeled phosphate to the reaction system containing the protein of the present invention and the substrate and measuring the radioactivity of the phosphate attached to the substrate.

Example 10: Rational Drug Design

10 The goal of rational drug design is to produce structural analogs of biologically active polypeptides of interest or of small molecules with which they interact, agonists, antagonists, or inhibitors. Any of these examples are used to fashion drugs which are more active or stable forms of the polypeptide or which enhance or interfere with the function of a polypeptide *in vivo*.

In one approach, the three-dimensional structure of a protein of interest, or of a protein-inhibitor 15 complex, is determined by x-ray crystallography, by computer modeling or, most typically, by a combination of the two approaches. Both the shape and charges of the polypeptide must be ascertained to elucidate the structure and to determine active site(s) of the molecule. Less often, useful information regarding the structure of a polypeptide is gained by modeling based on the structure of homologous proteins. In both cases, relevant structural information is used to design 20 efficient inhibitors. Useful examples of rational drug design include molecules which have improved activity or stability or which act as inhibitors, agonists, or antagonists of native peptides.

It is also possible to isolate a target-specific antibody, selected by functional assay, as described above, and then to solve its crystal structure. This approach, in principle, yields a pharmacore upon which subsequent drug design is based. It is possible to bypass protein crystallography 25 altogether by generating anti-idiotypic antibodies (anti-ids) to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-ids is expected to be an analog of the original receptor. The anti-id is then used to identify and isolate peptides from banks of chemically or biologically produced peptides. The isolated peptides then act as the pharmacore.

30 By virtue of the present invention, sufficient amount of polypeptide are made available to perform such analytical studies as X-ray crystallography. In addition, knowledge of the PRKG1 amino acid

sequence provided herein provides guidance to those employing computer modeling techniques in place of or in addition to x-ray crystallography.

Example 11: Identification of Other Members of the Signal Transduction Complex

Labeled PRKG1 is useful as a reagent for the purification of molecules with which it interacts. In one embodiment of affinity purification, PRKG1 is covalently coupled to a chromatography column. Cell-free extract derived from synovial cells or putative target cells is passed over the column, and molecules with appropriate affinity bind to PRKG1. PRKG1-complex is recovered from the column, and the PRKG1-binding ligand disassociated and subjected to N-terminal protein sequencing. The amino acid sequence information is then used to identify the captured molecule or to design degenerate oligonucleotide probes for cloning the relevant gene from an appropriate cDNA library.

In an alternate method, antibodies are raised against PRKG1, specifically monoclonal antibodies. The monoclonal antibodies are screened to identify those which inhibit the binding of labeled PRKG1. These monoclonal antibodies are then used therapeutically.

15 Example 12: Use and Administration of Antibodies, Inhibitors, or Antagonists

Antibodies, inhibitors, or antagonists of PRKG1 or other treatments and compounds that are limiters of signal transduction (LSTs), provide different effects when administered therapeutically. LSTs are formulated in a nontoxic, inert, pharmaceutically acceptable aqueous carrier medium preferably at a pH of about 5 to 8, more preferably 6 to 8, although pH may vary according to the characteristics of the antibody, inhibitor, or antagonist being formulated and the condition to be treated. Characteristics of LSTs include solubility of the molecule, its half-life and antigenicity/-immunogenicity. These and other characteristics aid in defining an effective carrier. Native human proteins are preferred as LSTs, but organic or synthetic molecules resulting from drug screens are equally effective in particular situations.

25 LSTs are delivered by known routes of administration including but not limited to topical creams and gels; transmucosal spray and aerosol; transdermal patch and bandage; injectable, intravenous and lavage formulations; and orally administered liquids and pills particularly formulated to resist stomach acid and enzymes. The particular formulation, exact dosage, and route of administration is determined by the attending physician and varies according to each specific situation.

30 Such determinations are made by considering multiple variables such as the condition to be treated, the LST to be administered, and the pharmacokinetic profile of a particular LST. Additional factors which are taken into account include severity of the disease state, patient's age,

weight, gender and diet, time and frequency of LST administration, possible combination with other drugs, reaction sensitivities, and tolerance/response to therapy. Long acting LST formulations might be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular LST.

- 5 Normal dosage amounts vary from 0.1 to 10^5 µg, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature; see U.S. Pat. Nos. 4,657,760; 5,206,344; or 5,225,212. Those skilled in the art employ different formulations for different LSTs. Administration to cells such as nerve cells necessitates delivery in a manner different from that to other cells such as vascular endothelial
10 cells.

It is contemplated that abnormal signal transduction, trauma, or diseases which trigger PRKG1 activity are treatable with LSTs. These conditions or diseases are specifically diagnosed by the tests discussed above, and such testing should be performed in suspected cases of viral, bacterial or fungal infections, allergic responses, mechanical injury associated with trauma, hereditary
15 diseases, lymphoma or carcinoma, or other conditions which activate the genes of lymphoid or neuronal tissues.

Example 13: Production of Non-human Transgenic Animals

Animal model systems which elucidate the physiological and behavioral roles of the PRKG1 are produced by creating nonhuman transgenic animals in which the activity of the PRKG1 is either
20 increased or decreased, or the amino acid sequence of the expressed PRKG1 is altered, by a variety of techniques. Examples of these techniques include, but are not limited to: 1) Insertion of normal or mutant versions of DNA encoding a PRKG1, by microinjection, electroporation, retroviral transfection or other means well known to those skilled in the art, into appropriately fertilized embryos in order to produce a transgenic animal or 2) homologous recombination of mutant or
25 normal, human or animal versions of these genes with the native gene locus in transgenic animals to alter the regulation of expression or the structure of these PRKG1 sequences. The technique of homologous recombination is well known in the art. It replaces the native gene with the inserted gene and hence is useful for producing an animal that cannot express native PRKG1s but does express, for example, an inserted mutant PRKG1, which has replaced the native PRKG1 in the
30 animal's genome by recombination, resulting in underexpression of the kinase. Microinjection adds genes to the genome, but does not remove them, and the technique is useful for producing an animal which expresses its own and added PRKG1, resulting in overexpression of the PRKG1.

One means available for producing a transgenic animal, with a mouse as an example, is as follows: Female mice are mated, and the resulting fertilized eggs are dissected out of their oviducts. The eggs are stored in an appropriate medium such as cesiumchloride M2 medium. DNA or cDNA encoding PRKG1 is purified from a vector by methods well known to the one skilled in the art.

- 5 Inducible promoters may be fused with the coding region of the DNA to provide an experimental means to regulate expression of the transgene. Alternatively or in addition, tissue specific regulatory elements may be fused with the coding region to permit tissue-specific expression of the transgene. The DNA, in an appropriately buffered solution, is put into a microinjection needle (which may be made from capillary tubing using a piper puller) and the egg to be injected is put in
10 a depression slide. The needle is inserted into the pronucleus of the egg, and the DNA solution is injected. The injected egg is then transferred into the oviduct of a pseudopregnant mouse which is a mouse stimulated by the appropriate hormones in order to maintain false pregnancy, where it proceeds to the uterus, implants, and develops to term. As noted above, microinjection is not the only method for inserting DNA into the egg but is used here only for exemplary purposes.

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Claims

1. A method of screening for therapeutic agents useful in the treatment of a disease comprised in a group of diseases consisting of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases in a mammal comprising the steps of
 - i) contacting a test compound with a PRKG1 polypeptide,
 - ii) detect binding of said test compound to said PRKG1 polypeptide.
- 10 2. A method of screening for therapeutic agents useful in the treatment of a disease comprised in a group of diseases consisting of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases in a mammal comprising the steps of
 - i) determining the activity of a PRKG1 polypeptide at a certain concentration of a test compound or in the absence of said test compound,
 - ii) determining the activity of said polypeptide at a different concentration of said test compound.
- 20 3. A method of screening for therapeutic agents useful in the treatment of a disease comprised in a group of diseases consisting of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases in a mammal comprising the steps of
 - i) determining the activity of a PRKG1 polypeptide at a certain concentration of a test compound,
 - ii) determining the activity of a PRKG1 polypeptide in the presence of a compound known to be a regulator of a PRKG1 polypeptide.

4. The method of any of claims 1 to 3, wherein the step of contacting is in or at the surface of a cell.
5. The method of any of claims 1 to 3, wherein the cell is in vitro.
6. The method of any of claims 1 to 3, wherein the step of contacting is in a cell-free system.
- 5 7. The method of any of claims 1 to 3, wherein the polypeptide is coupled to a detectable label.
8. The method of any of claims 1 to 3, wherein the compound is coupled to a detectable label.
9. The method of any of claims 1 to 3, wherein the test compound displaces a ligand which is first bound to the polypeptide.
- 10 10. The method of any of claims 1 to 3, wherein the polypeptide is attached to a solid support.
11. The method of any of claims 1 to 3, wherein the compound is attached to a solid support.
12. A method of screening for therapeutic agents useful in the treatment of a disease comprised in a group of diseases consisting of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases in a mammal comprising the steps of
15 i) contacting a test compound with a PRKG1 polynucleotide,
ii) detect binding of said test compound to said PRKG1 polynucleotide.
- 20 13. The method of claim 12 wherein the nucleic acid molecule is RNA.
14. The method of claim 12 wherein the contacting step is in or at the surface of a cell.
15. The method of claim 12 wherein the contacting step is in a cell-free system.
16. The method of claim 12 wherein polynucleotide is coupled to a detectable label.
17. The method of claim 12 wherein the test compound is coupled to a detectable label.
- 25 18. A method of diagnosing a disease comprised in a group of diseases consisting of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological

diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases in a mammal comprising the steps of

- 5 i) determining the amount of a PRKG1 polynucleotide in a sample taken from said mammal,
 - ii) determining the amount of PRKG1 polynucleotide in healthy and/or diseased mammals.
19. A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases in a mammal comprising a therapeutic agent which binds to a PRKG1 polypeptide.
20. A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases in a mammal comprising a therapeutic agent which regulates the activity of a PRKG1 polypeptide.
- 25 21. A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases in a mammal comprising a therapeutic agent which regulates the activity of a PRKG1 polypeptide, wherein said therapeutic agent is
- 30 i) a small molecule,
 - ii) an RNA molecule,
 - iii) an antisense oligonucleotide,
 - iv) a polypeptide,
 - v) an antibody, or
 - vi) a ribozyme.

22. A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases in a mammal comprising a PRKG1 polynucleotide.
- 5
23. A pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases in a mammal comprising a PRKG1 polypeptide.
- 10
24. Use of regulators of a PRKG1 for the preparation of a pharmaceutical composition for the treatment of a disease comprised in a group of diseases consisting of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases in a mammal.
- 15
25. Method for the preparation of a pharmaceutical composition useful for the treatment of a disease comprised in a group of diseases consisting of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases in a mammal comprising the steps of
- 20
- i) identifying a regulator of PRKG1,
- ii) determining whether said regulator ameliorates the symptoms of a disease comprised in a group of diseases consisting of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases in a mammal; and
- 25
- iii) combining of said regulator with an acceptable pharmaceutical carrier.
- 30
26. Use of a regulator of PRKG1 for the regulation of PRKG1 activity in a mammal having a disease comprised in a group of diseases consisting of cardiovascular diseases, respiratory

diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases.



Diagnostics and Therapeutics for Diseases Associated with Protein Kinase, cGMP-Dependent, Type I (PRKG1)

Abstract of Disclosure

The invention provides a human PRKG1 which is associated with cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases. The invention also provides assays for the identification of compounds useful in the treatment or prevention of cardiovascular diseases, respiratory diseases, dermatological diseases, endocrinological diseases, metabolic diseases, inflammation, gastroenterological diseases, cancer, hematological diseases, muscle-skeleton-diseases, neurological diseases and urological diseases. The invention also features compounds which bind to and/or activate or inhibit the activity of PRKG1 as well as pharmaceutical compositions comprising such compounds.



Fig. 1

SEQ ID NO: 1

AGGAAGCCTCAAGACGCCGGAGCAGCGGCAGGAAGGAGCCCCGGCAGC
CCGGAGGAGCATGGGCACCTTGCAGGGATTTACAGTACGCGCTCCAGGA
GAAGATCGAGGAGCTGAGGCAGCGGGATGCTCTCATCGACGAGCTGGA
GCTGGAGTTGGATCAGAAGGACGAACGTGATCCAGAAGCTGCAGAACGA
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Fig. 1 (continued)

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Fig. 1 (continued)

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Fig. 2

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Fig. 3

SEQ ID NO: 3

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Fig. 4

SEQ ID NO: 4

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Fig. 5

SEQ ID NO: 5

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24. März 2004

SEQUENCE LISTING

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